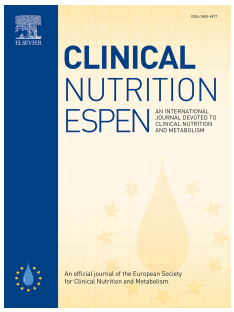

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Dietary adherence and disability evolution in multiple sclerosis: an exploratory 12-month prospective cohort study

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Short title: Dietary adherence in MS disease

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Abstract

Background & aims

Lifestyle factors may influence disability trajectories in multiple sclerosis (MS), yet prospective data linking sustained dietary adherence to objective clinical outcomes remain limited. Homocysteine, a metabolite involved in one-carbon metabolism, represents a biologically plausible mediator between nutrition and neurodegeneration, although its longitudinal clinical relevance in MS is uncertain.

This study aimed to investigate whether adherence to a structured dietary intervention is associated with longitudinal changes in plasma homocysteine levels and disability progression in patients with MS over 12 months.

Methods

In this prospective cohort study, 41 patients with MS were followed for 12 months. Dietary adherence was quantified using a structured follow-up scale (range 2–6). Plasma homocysteine levels and Expanded Disability Status Scale (EDSS) scores were assessed at baseline, 6 months, and 12 months. Associations between adherence, metabolic changes, and EDSS evolution were evaluated using correlation analyses and multivariate linear regression adjusting for age, sex, BMI, baseline EDSS, and treatment line.

Results

Baseline mean homocysteine was 10.91 ± 6.94 µmol/L and decreased to 8.24 ± 3.33 µmol/L at 6 months, remaining stable at 8.27 ± 2.42 µmol/L at 12 months. Between-group differences in homocysteine showed a trend toward significance at 12 months (ANOVA $p = 0.059$). Mean EDSS increased slightly from 1.14 to 1.23 in the overall cohort ($p > 0.05$). However, EDSS evolution differed according to dietary adherence (ANOVA p =

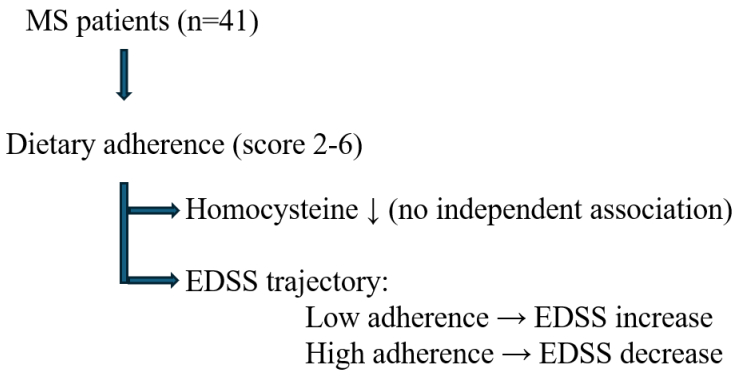
0.009): low-adherence patients showed a mean EDSS increase (+0.53 ± 0.72), whereas high-adherence patients showed a mean EDSS decrease (−0.56 ± 1.08). Given the low baseline EDSS, short follow-up, and small high-adherence subgroup, these changes should be interpreted as exploratory differences in short-term EDSS trajectory rather than confirmed clinical improvement and do not establish causality. Adherence score was inversely correlated with EDSS change ($r = -0.57$, $p = 0.0006$) and remained independently associated in multivariate analysis ($\beta = -0.45$, 95% CI −0.68 to −0.22, $p = 0.0005$).

Conclusions

Higher adherence to the dietary counselling programme was associated with a more favourable short-term disability trajectory over 12 months. However, causality cannot be inferred, and the clinical significance of these modest changes remains uncertain. Because the adherence score was not formally validated and may partly reflect broader health-related behaviours or engagement with care, and because the dietary intervention was individualized, the study cannot identify specific dietary components or dietary patterns associated with disability trajectories. These exploratory findings require confirmation in larger controlled studies using validated dietary assessment instruments.

Keywords: multiple sclerosis; dietary adherence; homocysteine; expanded disability status scale; lifestyle factors

GRAPHICAL ABSTRACT



1. INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated disorder of the central nervous system characterized by inflammatory demyelination and progressive neuroaxonal loss (1). Despite major advances in disease-modifying therapies (DMTs), long-term disability accumulation remains insufficiently controlled in a substantial proportion of patients (2, 3). While DMTs primarily target inflammatory activity, progression appears to result from complex interactions between immune mechanisms, neurodegeneration, metabolic dysfunction, and environmental influences (4). Identifying modifiable factors associated with disability trajectories therefore remains a clinical priority.

In addition to genetic susceptibility, lifestyle factors contribute to MS risk and possibly disease course (5). Smoking, obesity, and vitamin D deficiency have been consistently associated with worse outcomes (6-8). Increasing attention has focused on diet as a potentially modifiable determinant of inflammatory tone, metabolic homeostasis, and neurodegeneration (9, 10). However, robust longitudinal data linking sustained dietary adherence to objective clinical endpoints in MS remain limited.

One biological pathway potentially linking diet and neurodegeneration involves one-carbon metabolism and its central intermediate, homocysteine (11). Homocysteine is regulated by folate- and vitamin B12-dependent pathways and influences oxidative stress, endothelial function, excitotoxicity, and methylation processes (12, 13). Elevated homocysteine levels have been associated with vascular and neurodegenerative disorders (14), and several studies suggest that patients with MS may exhibit higher circulating concentrations compared with healthy controls (15-17). Nonetheless, the relationship between homocysteine levels and longitudinal disability progression in MS remains unclear.

From a clinical perspective, the Expanded Disability Status Scale (EDSS) remains the most widely used measure of disability in MS (18). Even modest changes over a one-year period may reflect meaningful shifts in disease trajectory, particularly in patients at risk of progression. Yet few prospective studies have simultaneously evaluated sustained dietary adherence, metabolic biomarkers, and EDSS evolution.

In this context, we conducted a 12-month prospective study to investigate whether adherence to a structured dietary intervention is associated with longitudinal changes in plasma homocysteine levels and disability progression in patients with MS. We hypothesized that sustained dietary adherence would be associated with reduced homocysteine concentrations and a more favourable EDSS trajectory, independently of baseline clinical characteristics and treatment exposure.

2. MATERIAL & METHODS

2.1. Study Design and Study Population

This was a 12-month prospective observational cohort study conducted in patients with clinically definite multiple sclerosis (MS) followed at a tertiary neurology center. Patients were recruited consecutively from the specialized multiple sclerosis outpatient clinic of the Neurology Department at the Complejo Asistencial Universitario de Salamanca, which provides care for approximately 450 patients with MS in the province. The study cohort therefore represents roughly 10% of the local MS population. Although the sample size was modest, it reflects a relatively homogeneous population from the same geographic region and shared dietary environment, which may reduce variability in dietary patterns and lifestyle factors. Baseline demographic and clinical variables available for analysis included age, sex, BMI, baseline EDSS and treatment line. Other potentially relevant variables, including MS phenotype, relapse activity during follow-

up, corticosteroid exposure, smoking status, vitamin or nutritional supplementation, socioeconomic status, and standardized physical activity measures, were not systematically available for all participants and were therefore not included in the primary multivariate models. The sex distribution in the cohort was consistent with the known female predominance of MS. Inclusion criteria were diagnosis of MS according to established diagnostic criteria; age ≥18 years, and availability of baseline and follow-up clinical and laboratory assessments. No formal sample size calculation was performed, as the study was designed as an exploratory prospective cohort conducted in routine clinical practice. The sample size was therefore determined by the number of eligible patients who completed baseline and follow-up assessments. As a result, analyses should be interpreted as exploratory, particularly subgroup, interaction, and homocysteine-related analyses. Patients were evaluated at baseline, 6 months, and 12 months. The study was conducted in accordance with the Declaration of Helsinki, and all participants provided written informed consent.

2.2. Dietary Intervention and Adherence Assessment

Participants received individualized dietary counselling delivered in the nutrition clinic as part of routine clinical practice. Although menus were personalized, all participants received the same core nutritional framework. The dietary plan was adapted to each patient's baseline dietary habits, food preferences, weight objective, clinical/metabolic profile, physical activity, work schedule, family routine, and practical circumstances, but always within predefined nutritional criteria. At the first nutrition visit, participants underwent a 24-hour dietary recall and completed a food-frequency questionnaire. They were also given a dietary diary to record all food intake during the following week, which was reviewed at the second visit. Weight, height, wrist circumference, and physical activity were assessed, and total energy expenditure was

estimated to guide weight-related objectives when appropriate. At the second visit, the first individualized diet was prescribed. In subsequent visits, weight was monitored, adherence to the previous dietary plan was evaluated, and the diet was adjusted when needed to improve adherence, optimize results, or increase menu variety while maintaining the general study criteria. The frequency of counselling visits was individualized according to the presence or absence of a weight-change objective, baseline dietary habits, and patient availability, ranging from weekly to monthly visits. The common dietary targets were: (i) to maximize vegetable intake, with at least one daily serving and the greatest possible variety of vegetables and preparation methods; (ii) to establish a structured intake of carbohydrate-rich foods, including legumes weekly and pasta, rice, couscous, or potato-based dishes approximately 2–3 times per week when not contraindicated, together with individualized bread portions; (iii) to ensure regular intake of lean, non-processed protein sources, including lean meats, fish, with attention to oily fish, and eggs, generally corresponding to 1–2 protein servings per day; and (iv) to include fruit and dairy products according to individual tolerance and dietary needs. Participants were advised to eliminate or reduce to a minimum nutritionally unfavourable foods, including processed foods, sugar-rich products, pastries, biscuits, processed meats and sausages, sugar-sweetened beverages, alcoholic drinks, and culinary preparations that substantially increased the caloric load of the diet. The dietary counselling also emphasized limiting components potentially associated with systemic inflammation, including saturated animal fats, industrial trans fats, red meat, highly refined carbohydrates, excessive salt intake, and highly processed foods. Additional details on the dietary assessment protocol, food-frequency questionnaire, dietary diary, follow-up visits, and adherence categorization are provided in Supplementary Table S2. Because dietary counselling was individualized and dietary intake was not quantified using a

validated nutrient-level assessment, the study was not designed to isolate the contribution of specific foods, nutrients, or dietary patterns.

Dietary adherence was assessed using a pragmatic follow-up score developed for monitoring compliance within this research project. The score was not intended to provide a quantitative estimate of nutrient intake, nor was it designed as a validated clinical dietary scale. Adherence was assessed at two predefined intervals, corresponding to the first and second semesters of the 12-month follow-up period. At each interval, adherence was rated using a three-level ordinal scale based on dietary follow-up interviews and review of compliance with the prescribed dietary plan: poor adherence, defined as poor compliance in all or most consultations, was scored as 1; irregular adherence, defined as approximately half of the consultations suggesting good compliance, was scored as 2; and good adherence, defined as good compliance in all or most consultations, was scored as 3. The total adherence score was calculated as the sum of the two semester scores, yielding a cumulative score ranging from 2 to 6. Clinically, this score should be interpreted as an ordinal measure of sustained adherence to the dietary counselling programme over time. Lower values indicate poor or inconsistent adherence, whereas higher values indicate more sustained adherence across the follow-up period. The score was applied systematically within the study; however, it has not been externally validated, and formal inter-rater reproducibility was not assessed. Patients with higher adherence may also differ in broader health-related behaviours, including motivation, health literacy, treatment adherence, physical activity, smoking avoidance, supplement use, and engagement with medical care. Therefore, the observed association may partly reflect a broader health-behaviour profile rather than a direct effect of dietary adherence on disability evolution. Therefore, analyses involving this score should be interpreted as exploratory and hypothesis-generating. In addition to the cumulative

adherence score, adherence trajectories were explored by comparing the adherence rating obtained during the first semester with that obtained during the second semester. Patients were classified as having improved adherence when the second-semester score was higher than the first-semester score, stable adherence when both scores were unchanged, and worsened adherence when the second-semester score was lower than the first-semester score. This trajectory analysis was considered exploratory and was used to complement the summed adherence score.

2.3. Clinical Assessment

Neurological disability was assessed using the Expanded Disability Status Scale (EDSS) at baseline, 6 months, and 12 months. EDSS evaluations were performed during routine neurological follow-up by neurologists blinded to patients' dietary adherence category. Clinical relapse occurrence during follow-up was reviewed, and no relapses were recorded among patients who completed the 12-month follow-up. Therefore, the EDSS assessments included in the longitudinal analysis were not obtained in the context of documented relapses during the study period. The primary clinical endpoint was change in EDSS from baseline to 12 months.

2.4. Laboratory Measurements

Fasting blood samples were collected at baseline, 6 months, and 12 months. Plasma homocysteine levels were measured using standard clinical laboratory methods. The primary metabolic endpoint was change in plasma homocysteine from baseline to 12 months. Some biochemical parameters were available only in a subset of patients due to routine clinical testing and were therefore not available for all patients at all time points. These variables were analysed descriptively and were not considered primary mechanistic endpoints. Secondary laboratory parameters included: haemoglobin, glucose, uric acid,

creatinine, calcium, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, albumin, folate, thyroid-stimulating hormone (TSH), vitamin D and vitamin B12. Longitudinal changes were calculated as follow-up minus baseline values.

2.5. Treatment Exposure

Disease-modifying therapies (DMTs) at baseline were categorized as: first-line therapy; second-line therapy; and no treatment. Treatment line was included as a covariate in multivariate models.

2.6. Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequencies and percentages. Within-subject changes between baseline and follow-up were analysed using paired t-tests. Between-group comparisons across adherence categories were performed using one-way analysis of variance (ANOVA). Pearson correlation coefficients were calculated to assess the association between adherence score (continuous), Δhomocysteine, and ΔEDSS. Multivariate linear regression models were constructed to identify independent predictors of 12-month EDSS change. The primary multivariate model included adherence score, age, sex, BMI, baseline EDSS, and treatment line. These covariates were selected a priori based on clinical relevance and availability across the cohort. Additional potential confounders, including MS phenotype, relapse activity, corticosteroid use, smoking, physical activity, supplementation, socioeconomic status, and validated baseline diet quality measures, were not included because they were not systematically available or were incomplete. A separate regression model was constructed to evaluate predictors of homocysteine change, adjusting for: age, sex, BMI, baseline homocysteine and baseline EDSS. Interaction between adherence score and treatment line was tested by including an

237 interaction term in the regression model. All analyses were two-sided, and p-values < 0.05
238 were considered statistically significant. Given the exploratory nature and modest sample
239 size of the study, emphasis was placed not only on p-values but also on effect estimates
240 and 95% confidence intervals as indicators of precision. Non-significant findings,
241 particularly in subgroup and homocysteine analyses, were interpreted cautiously because
242 of the possibility of type II error. As an exploratory analysis, adherence trajectories were
243 analysed descriptively by comparing first- and second-semester adherence scores.
244 Because only one patient showed improved adherence, stable and improved trajectories
245 were combined for the comparison of EDSS change with the worsened-adherence group.

246 **3. RESULTS**

247 **3.1. Study population**

248 A total of 41 patients with multiple sclerosis completed the 12-month follow-up and
249 were included in the longitudinal analysis. Baseline demographic and clinical
250 characteristics are summarized in Table 1. The mean age was 40 ± 8.76 years, and 31
251 participants were female (75.6%). The mean baseline EDSS score was 1.14 ± 1.58.
252 Regarding treatment exposure, 58.5% were receiving first-line disease-modifying therapy
253 (24/41) and 39.0% second-line therapy (16/41). One patient (2.5%) was untreated. No
254 significant differences in age, sex distribution, baseline EDSS, BMI, or treatment line
255 were observed between dietary adherence groups at baseline (all p > 0.05).

256 **3.2. Dietary adherence over 12 months**

257 Dietary adherence was quantified using a pragmatic ordinal follow-up score
258 integrating adherence during the first and second semesters of the study period. Total
259 scores ranged from 2 to 6, with higher scores indicating more sustained adherence to the
260 dietary counselling programme. For descriptive and stratified analyses, scores of 2–3

were interpreted as low adherence, a score of 4 as moderate adherence, and scores of 5–6 as high adherence. These categories should be interpreted as exploratory adherence strata rather than as externally validated clinical cut-offs. At the end of follow-up: 24.2% of patients were classified as having high adherence (score 5–6); 30.3% as having moderate adherence (score 4), and 45.5% as having low adherence (score 2–3) (Fig. 1). In the exploratory trajectory analysis, 20 patients showed stable adherence, 1 patient improved adherence, and 12 patients showed worsened adherence between the first and second semesters. Thus, among patients with available trajectory data, 21/33 patients (63.6%) had stable or improved adherence, whereas 12/33 patients (36.4%) had worsened adherence during follow-up.

3.3. Longitudinal changes in plasma homocysteine

In the overall cohort, mean plasma homocysteine concentration decreased significantly from baseline (10.91 ± 6.94 μmol/L) to 6 months (8.24 ± 3.33 μmol/L; $p = 0.012$) and remained significantly lower at 12 months than at baseline (8.27 ± 2.42 μmol/L; $p = 0.015$). No significant difference was observed between 6 and 12 months ($p > 0.05$) (Fig. 2).

When patients were stratified by adherence category, the mean 12-month change in homocysteine was −3.10 ± 2.04 μmol/L in the high-adherence group, −0.03 ± 3.21 μmol/L in the moderate-adherence group, and −2.99 ± 3.46 μmol/L in the low-adherence group. Between-group differences showed a trend toward significance (ANOVA $p = 0.059$) (Fig. 3).

In multivariate linear regression adjusting for age, sex, BMI, baseline homocysteine, and baseline EDSS, total adherence score was not independently associated with homocysteine change ($\beta = -0.47$, 95% CI −1.09 to 0.16, $p = 0.138$). However, the

confidence interval was relatively wide and included potentially relevant negative associations; therefore, this non-significant finding should be interpreted cautiously considering the limited sample size.

3.4. Disability evolution (EDSS)

No clinical relapses were recorded among patients who completed the 12-month follow-up. Therefore, changes in EDSS during the study period were not attributable to documented relapse-related disability worsening. During the 12-month follow-up, the mean EDSS score increased slightly from 1.14 to 1.23, corresponding to an approximate absolute change of +0.13). This overall change did not reach statistical significance (paired t-test $p > 0.05$) (Fig. 4).

EDSS evolution differed according to dietary adherence (Fig. 5). The low-adherence group showed a mean EDSS increase of $+0.53 \pm 0.72$ ($n = 15$); the moderate-adherence group showed no mean change 0.00 ± 0.47 ($n = 10$); and the high-adherence group showed a mean EDSS decrease of -0.56 ± 1.08 ($n = 8$). The between-group comparison was statistically significant (ANOVA $p = 0.009$). Because baseline EDSS was low in the overall cohort and the high-adherence subgroup was small, these subgroup differences should be interpreted cautiously as exploratory changes in EDSS trajectory rather than as confirmed disability improvement.

We also explored EDSS change according to adherence trajectory (Supplementary Fig. 1). Because only one patient improved adherence, patients with stable or improved adherence were analysed together and compared with those whose adherence worsened. Mean EDSS change was $+0.143 \pm 0.839$ in the stable/improved group and $+0.0417 \pm 0.940$ in the worsened-adherence group. This difference was not statistically significant ($p = 0.752$). Therefore, this exploratory trajectory analysis did not show a significant

difference in EDSS change between patients with stable/improved adherence and those with worsened adherence.

When adherence was analysed as a continuous variable, it was inversely correlated with EDSS change ($r = -0.57$, $p = 0.0006$), indicating that higher adherence was associated with lower EDSS change over 12 months. In multivariate linear regression adjusted for baseline EDSS, age, sex, BMI, and treatment line, adherence score remained independently associated with EDSS evolution ($\beta = -0.45$, 95% CI −0.68 to −0.22, $p = 0.0005$). This association should be interpreted as exploratory and does not imply causality. No interaction was observed between treatment line and adherence ($p = 0.419$) (Fig. 6).

3.5. Secondary metabolic parameters

Changes in haemoglobin, glucose, urate, creatinine, calcium, cholesterol, HDL, LDL, triglycerides, albumin, folate, TSH, vitamin D and vitamin B12 levels are summarized in Supplementary Table S1. None of these parameters independently predicted EDSS change in multivariate analysis (all $p > 0.05$).

4. DISCUSSION

In this 12-month prospective cohort study, higher dietary adherence was associated with a more favourable EDSS trajectory, after adjustment for baseline EDSS, age, sex, BMI, and treatment line. However, because of the observational design, causality cannot be inferred. These findings should therefore be interpreted as an association between adherence and short-term EDSS evolution rather than evidence that dietary adherence caused disability improvement. In contrast, although plasma homocysteine levels decreased during follow-up, adherence-dependent differences in homocysteine reduction did not reach statistical significance after multivariable adjustment. These findings

suggest that sustained dietary adherence may be linked to clinically meaningful disability trajectories, independently of conventional therapeutic exposure.

4.1. Dietary adherence and disability evolution

The main clinical finding of this study was an association between higher dietary adherence and a more favourable EDSS trajectory over 12 months. However, the clinical meaning of the observed EDSS changes should be interpreted cautiously and causality cannot be inferred because of the observational design. Baseline disability was low in this cohort, and the overall mean EDSS change during follow-up was small and not statistically significant. Although EDSS evolution differed across adherence categories, the high-adherence subgroup was small, which limits the precision and generalizability of this estimate. In addition, small EDSS changes over a 12-month period, particularly in patients with low baseline EDSS scores, may reflect measurement variability, regression to the mean, or transient functional fluctuations rather than confirmed disability change. Therefore, these results should be viewed as exploratory evidence of an association between dietary adherence and short-term EDSS evolution, rather than proof of clinically meaningful disability improvement.

The trajectory analysis provides complementary information to the summed adherence score. While the summed score reflects cumulative adherence over the full 12-month period, the trajectory analysis distinguishes patients who maintained or improved adherence from those whose adherence declined. In this cohort, adherence was stable or improved in 63.6% of patients with available trajectory data and worsened in 36.4%. However, EDSS change did not differ significantly between stable/improved and worsened-adherence trajectory groups. This may reflect the limited sample size, the small number of patients with improved adherence, the availability of only two adherence

assessments, or the possibility that cumulative adherence better captures the relevant exposure than direction of change alone. Therefore, the trajectory analysis should be interpreted as exploratory and complementary to the main summed-score analysis.

Our results are consistent with emerging observational evidence suggesting that lifestyle factors may influence disability progression in MS. Large cohort studies have shown that healthier lifestyle behaviours (including diet quality, physical activity, and smoking avoidance) are associated with lower disability levels and better quality of life (19, 20). Although randomized dietary trials remain limited and heterogeneous, some interventional studies have reported improvements in fatigue, quality of life, and functional measures following structured dietary interventions (21, 22). However, robust prospective data directly linking dietary adherence to EDSS trajectories are scarce, and our findings contribute to this gap.

The adherence score may not reflect dietary adherence alone. Patients with higher adherence may also differ in broader health-related behaviours, including motivation, health literacy, treatment adherence, physical activity, smoking avoidance, supplement use, and engagement with medical care. Therefore, the observed association may partly reflect a broader health-behaviour profile rather than a direct effect of dietary adherence on disability evolution.

Importantly, the observed association in our study was independent of treatment line and baseline disability. This suggests that dietary adherence may represent a complementary factor influencing disability evolution rather than simply reflecting differences in disease severity or therapeutic intensity. Given that progression in MS involves mechanisms beyond focal inflammatory activity (including neurodegeneration,

mitochondrial dysfunction, and chronic microglial activation) (3, 4), modifiable lifestyle factors may plausibly exert additive effects alongside disease-modifying therapies.

Although the magnitude of EDSS change over one year was modest at the cohort level, stratified analysis revealed divergent trajectories according to adherence category. Even relatively small EDSS changes over short intervals may carry prognostic relevance, particularly in early disease stages (2). However, because baseline disability was low, follow-up was short, and the high-adherence subgroup was small, the observed EDSS differences should be interpreted as exploratory short-term trajectories rather than confirmed clinically meaningful disability improvement. In addition, because the intervention was individualized, our findings cannot determine which specific dietary components or dietary patterns, if any, were associated with the observed disability trajectories. The results should therefore be interpreted in relation to global adherence to the dietary counselling programme rather than to any individual food group, nutrient, or dietary pattern. Moreover, given the low baseline disability values and the very small high-adherence subgroup, the clinical relevance and stability of the observed disability differences remain uncertain. These findings should therefore be interpreted as exploratory short-term trajectories rather than evidence of clinically meaningful disability improvement.

4.2. Homocysteine as a metabolic mediator: partial support

Homocysteine levels decreased significantly during follow-up, particularly within the first six months, and this reduction was maintained at 12 months. Importantly, the pattern of homocysteine change across adherence groups was not dose-dependent. Both the high- and low-adherence groups showed reductions in homocysteine, whereas the moderate-adherence group showed little change. This inconsistent pattern weakens the

404 interpretation that dietary adherence was the main driver of homocysteine reduction.
405 Therefore, although homocysteine decreased in the overall cohort, our data do not support
406 homocysteine reduction as a clear adherence-dependent mechanism linking dietary
407 counselling to EDSS evolution.

408 Elevated homocysteine levels have been reported in patients with MS in several meta-
409 analyses (15-17). Mechanistically, homocysteine has been linked to oxidative stress,
410 endothelial dysfunction, excitotoxicity, and impaired methylation (11, 12). These
411 processes overlap with pathways implicated in neurodegeneration and progressive MS.
412 Nevertheless, longitudinal studies evaluating homocysteine as a predictor of disability
413 progression remain limited and inconsistent. Our findings suggest that homocysteine may
414 represent only one component of a broader metabolic network rather than a singular
415 mediator of disability change.

416 Several explanations merit consideration. First, the sample size may have limited
417 statistical power to detect adherence-dependent differences in homocysteine change,
418 particularly in subgroup analyses. Although homocysteine decreased significantly in the
419 overall cohort, between-group differences according to adherence showed only a trend
420 toward significance, and the adjusted association between adherence score and
421 homocysteine change did not reach statistical significance. However, the confidence
422 interval around this estimate was relatively wide, indicating limited precision. Therefore,
423 type II error cannot be excluded, and the absence of an independent association should
424 not be interpreted as definitive evidence that dietary adherence is unrelated to
425 homocysteine change. Second, metabolic modulation induced by diet likely involves
426 multiple interacting pathways, including inflammatory, mitochondrial, and epigenetic
427 mechanisms not captured by homocysteine alone. Third, disability evolution in MS is
428 multifactorial, and a single circulating biomarker may not adequately reflect complex

neurobiological processes. Fourth, the present study was not designed to establish a mechanistic pathway linking dietary adherence, one-carbon metabolism, and EDSS evolution. Folate and vitamin B12, which are directly involved in homocysteine metabolism, were available only in limited patient subsets. This prevents robust assessment of whether homocysteine changes reflected changes in these cofactors, supplementation, or other metabolic determinants. Therefore, homocysteine should be interpreted as an exploratory metabolic marker in this cohort rather than as a demonstrated mediator.

Nevertheless, the sustained reduction in homocysteine observed in our cohort supports the biological plausibility of diet-induced metabolic modulation and aligns with broader literature linking one-carbon metabolism to neurodegenerative processes (12).

4.3. Potential mechanisms

If the observed association reflects, at least in part, a biological effect of dietary adherence, several mechanisms could be hypothesized. Dietary patterns may be associated with systemic inflammatory tone (9); oxidative stress burden (10); endothelial and microvascular function (13); mitochondrial efficiency (3); epigenetic regulation through methylation pathways (23); and gut microbiota composition and immune signaling (24). However, these pathways were not directly tested in the present study and should be considered mechanistic hypotheses rather than demonstrated mediators.

These pathways may converge on neuroaxonal resilience and remyelination capacity, potentially influencing disability trajectories independently of relapse suppression. The absence of interaction with treatment line in our study suggests that dietary adherence may exert additive rather than competing effects with pharmacological therapies.

4.4. Secondary metabolic parameters

Although changes in several metabolic markers were observed during follow-up, none independently predicted EDSS change. This supports the hypothesis that the association between dietary adherence and disability evolution may reflect an integrated biological effect rather than dependence on a single laboratory parameter.

4.5. Strengths and limitations

Strengths of this study include its prospective design, simultaneous assessment of clinical and metabolic endpoints, stratified and continuous adherence analyses, and multivariate adjustment including treatment line. EDSS assessments were performed by blinded neurologists, reducing potential assessment bias.

Several limitations should be acknowledged. First, the modest sample size limited statistical power, particularly for subgroup analyses, interaction testing, and homocysteine-related analyses. No formal sample size calculation was performed, and the study was not powered to detect small-to-moderate metabolic effects or to formally evaluate homocysteine as a mediator between dietary adherence and EDSS evolution. The high-adherence subgroup was small, which limits the precision and generalizability of estimates derived from stratified analyses. Therefore, non-significant findings, especially those related to homocysteine change and secondary metabolic parameters, should be interpreted cautiously because type II error cannot be excluded. Larger studies with predefined sample size calculations are needed to confirm these associations and to clarify whether homocysteine is a mediator, marker, or unrelated metabolic correlate of disability evolution.

Second, the dietary intervention was conducted in routine clinical practice and consisted of individualized dietary counselling based on shared nutritional principles rather than a single standardized dietary protocol. Although this reflects real-world

clinical practice, it also introduces heterogeneity in the dietary intervention received by each participant. Because dietary counselling was individualized and detailed nutrient-level intake was not available, the study cannot identify which specific dietary components, nutrients, foods, or dietary patterns were associated with disability evolution.

Third, the mechanistic interpretation of homocysteine changes is also limited by incomplete availability of several laboratory variables directly involved in one-carbon metabolism. Although plasma homocysteine was available for most participants, folate and vitamin B12 were measured only in limited subsets and with variable availability across time points, because these parameters were obtained as part of routine clinical testing rather than a standardized biomarker protocol. Therefore, folate and vitamin B12 could not be robustly included in longitudinal or multivariate models, and we cannot determine whether the observed reduction in homocysteine was mediated by changes in folate status, vitamin B12 status, supplementation, dietary intake, renal function, or other metabolic pathways. For this reason, the homocysteine results should be interpreted as exploratory and descriptive, and not as evidence of a defined biochemical mechanism linking dietary adherence to EDSS evolution

Fourth, adherence was assessed using a pragmatic clinical follow-up score rather than a validated quantitative dietary assessment instrument. The dietary adherence score was a pragmatic monitoring tool developed for this research project and was not an externally validated dietary assessment instrument. Although it was based on predefined ordinal categories and applied systematically during follow-up, it partly relied on dietary interviews and perceived adherence, and formal inter-rater reproducibility was not assessed. The score should therefore be interpreted as a global measure of adherence to the counselling programme rather than as a quantitative measure of nutrient intake.

Consequently, the associations observed between adherence score and EDSS evolution should be considered exploratory and hypothesis-generating.

Fifth, although no clinical relapses were recorded among patients who completed the 12-month follow-up, relapse activity and corticosteroid exposure were not collected as detailed standardized variables beyond routine clinical follow-up. Similarly, the exact timing of EDSS assessment in relation to transient neurological symptoms or intercurrent events was not available. Therefore, although the available data suggest that EDSS changes were not driven by documented relapses, residual uncertainty related to relapse-independent fluctuations or steroid exposure cannot be completely excluded.

Sixth, residual confounding cannot be excluded. Although the main analyses were adjusted for age, sex, BMI, baseline EDSS, and treatment line, several potentially relevant confounders were not systematically available or could not be included in the multivariate models. These include MS phenotype, relapse activity during follow-up, corticosteroid use, smoking status, standardized physical activity measures, vitamin or nutritional supplementation, socioeconomic status, and validated baseline diet quality. Baseline dietary habits were assessed using dietary recall, food-frequency information, and broad food-group categories, but these data did not constitute a validated comprehensive diet quality index. Physical activity was considered during nutritional counselling to estimate energy expenditure and adapt diet prescription, but it was not collected as a standardized longitudinal exposure. Similarly, serum vitamin D, vitamin B12, and folate were available only in patient subsets, and supplement use was not systematically recorded. Therefore, the association between dietary adherence and EDSS evolution may be partly influenced by unmeasured lifestyle, clinical, or socioeconomic factors. In addition, patients with higher adherence to dietary counselling may also have differed in treatment adherence, motivation, health literacy, engagement with care, physical activity, smoking behaviour,

supplement use, or other health-related behaviours that were not systematically measured. Therefore, the observed association cannot be attributed to diet alone and may partly reflect a broader health-behaviour or care-engagement profile.

Seventh, the clinical interpretation of EDSS changes should be cautious. The interpretation of EDSS changes is limited by the low baseline EDSS scores, the short 12-month follow-up, and the small number of patients in the high-adherence subgroup. The study was not designed or powered to evaluate confirmed disability progression or confirmed disability improvement. Therefore, the observed EDSS differences should not be interpreted as definitive evidence of clinically meaningful neurological improvement. Longer follow-up and larger controlled cohorts are required to determine whether sustained dietary adherence is associated with confirmed disability outcomes. This uncertainty is particularly relevant because the high-adherence subgroup was very small and baseline disability values were low, limiting both the precision and clinical interpretation of the observed EDSS differences.

Finally, the observational design precludes causal inference. Although higher dietary adherence was associated with a more favourable EDSS trajectory, this association should not be interpreted as evidence that dietary counselling caused disability improvement. The findings should be considered exploratory and require confirmation in larger controlled studies with longer follow-up, validated dietary assessment tools, objective adherence biomarkers, systematic recording of relapses and corticosteroid exposure, and predefined confirmed disability outcomes.

4.6. Clinical implications

These findings suggest that adherence to dietary counselling may be associated with short-term disability trajectories in multiple sclerosis. However, the adherence score was

not formally validated and may partly reflect broader health-related behaviours, treatment adherence, or engagement with care rather than dietary adherence alone. In addition, because the dietary intervention was individualized, this study cannot determine which specific dietary components or dietary patterns were associated with the observed trajectories. From a clinical perspective, the observed disability differences should be interpreted cautiously, as their clinical relevance remains uncertain in the context of low baseline disability, short follow-up, and the very small high-adherence subgroup. Therefore, these findings should be considered exploratory and should not be interpreted as evidence that dietary counselling causes clinically meaningful disability improvement or as support for specific dietary recommendations.

5. CONCLUSION

In this exploratory 12-month prospective cohort, higher adherence to the dietary counselling programme was associated with a more favourable short-term disability trajectory. However, the dietary adherence score was not formally validated and may partly reflect broader health-related behaviours or engagement with care rather than dietary adherence alone. Because the intervention was individualized, the study cannot determine which specific dietary components, nutrients, or dietary patterns were associated with the observed trajectories. Given the modest sample size, low baseline disability values, small high-adherence subgroup, potential residual confounding, and observational design, these findings should be considered hypothesis-generating and require confirmation in larger controlled studies with longer follow-up, validated dietary assessment instruments, objective adherence biomarkers, and predefined confirmed disability outcomes.

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We would like to acknowledge all study participants that contributed to this study.

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Author's contribution

The authors' responsibilities were as follows - DGG: Methodology, formal analysis, data curation, conceptualization; LSA: Formal analysis, writing-original draft; YEM: Conceptualization, data curation; AVC: Writing-original draft, supervision, formal analysis, conceptualization and approbation the final manuscript.

Conflict of Interest

The authors declare that they have no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to patient privacy considerations and institutional data protection regulations. The data contain sensitive clinical information that cannot be fully anonymized without compromising confidentiality. De-identified data may be made available from the corresponding author upon reasonable request, subject to approval by the institutional ethics committee and in accordance with applicable data protection legislation.

All patients gave their (written) informed consent to participate in the study. All research was carried out in accordance with relevant guidelines and regulations.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to improve English edition of the manuscript and detect minor technical issues. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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Figure Legends

Fig. 1. Distribution of dietary adherence scores over the 12-month follow-up. Bar graph illustrating the proportion of patients according to total dietary adherence score.

Adherence was assessed at two time points (first and second semester) using a three-level scale (1 = poor adherence, 2 = irregular adherence, 3 = good adherence). The total adherence score represents the sum of both assessments (range 2–6) and reflects cumulative dietary adherence during the study period. Patients were subsequently categorized into low (scores 2–3), moderate (score 4), and high adherence (scores 5–6) groups for stratified analyses

Fig. 2. Longitudinal changes in plasma homocysteine concentration over 12 months. Bar graphs illustrate mean plasma homocysteine levels (μmol/L) at baseline, 6 months, and 12 months in the overall cohort. Error bars represent standard deviation.

Fig. 3. Change in plasma homocysteine according to dietary adherence. Box-and-whisker plots illustrate the variation in plasma homocysteine levels (12 months – baseline) stratified by adherence category. Boxes represent the interquartile range (IQR), horizontal lines indicate medians and whiskers represent the range excluding outliers. The individual data points are shown to improve transparency given the small subgroup sizes, particularly in the high-adherence group. Negative values indicate reduction in EDSS scores.

Fig. 4. Longitudinal changes in Expanded Disability Status Scale (EDSS) score over 12 months. Bar graphs illustrate mean EDSS values at baseline, 6 months, and 12 months in the overall cohort. Error bars represent standard deviation.

Fig. 5. Change in EDSS score according to dietary adherence. Box-and-whisker plots illustrate the variation in EDSS score (12 months – baseline) stratified by adherence category. Boxes represent the interquartile range (IQR), horizontal lines indicate medians and whiskers represent the range excluding outliers. The individual data points are shown to improve transparency given the small subgroup sizes,

694 particularly in the high-adherence group. Negative values indicate reduction in EDSS
695 scores.

696 **Fig. 6.** Multivariate linear regression model for 12-month EDSS change.
697 Coefficient plot showing β estimates and 95% confidence intervals for predictors included
698 in the adjusted regression model. The vertical reference line represents no association (β
699 = 0). Adherence score remained independently associated with disability evolution.

700 **Supplementary Fig. 1. (A)** Adherence trajectories over time. Distribution of adherence
701 trajectories between the first and second semesters. Patients were classified as improved,
702 stable, or worsened according to the direction of change in semester adherence scores.
703 **(B)** Change in EDSS score according to adherence trajectory.
704 Box-and-whisker plots illustrate the variation in EDSS score (12 months – baseline)
705 stratified by adherence category. Because only one patient showed improved adherence,
706 stable and improved trajectories were combined and compared with worsened adherence
707 Boxes represent the interquartile range (IQR), horizontal lines indicate medians and
708 whiskers represent the range excluding outliers. The individual data points are shown to
709 improve transparency given the small subgroup sizes, particularly in the high-adherence
710 group. Negative values indicate reduction in EDSS scores.

711

712 **Table 1.**

713 Baseline demographic and clinical characteristics of the study population

Variable	Total cohort (n = 41)
Age, years	40 ± 8.76 (n = 41)
Female sex, n (%)	31 (75.6%)
BMI, kg/m²	25.63 ± 5.26
Baseline EDSS	1.14 ± 1.58
EDSS range	0–6
First-line DMT, n (%)	24 (58.5%)
Second-line DMT, n (%)	16 (39.0%)
No DMT, n (%)	1 (2.5%)
Plasma homocysteine, µmol/L (n)	10.91 ± 6.94 (n = 41)
Vitamin B12, pg/mL (n)	445.4 ± 179.5 (n = 10)
Folate, ng/mL (n)	7.6 ± 3.72 (n = 9)
Albumin, g/dL (n)	4.71 ± 0.30 (n = 34)
Total cholesterol, mg/dL (n)	189.2 ± 28.94 (n = 35)
LDL cholesterol, mg/dL (n)	114.8 ± 24.6 (n = 18)
HDL cholesterol, mg/dL (n)	62.2 ± 17.2 (n = 18)
Vitamin D, ng/mL (n)	27.3 ± 8.87 (n = 24)
Serum iron, µg/dL (n)	78.6 ± 24.89 (n = 19)
Glucose, mg/dL (n)	81.8 ± 8.71 (n = 35)
Total proteins, g/dL (n)	7.07 ± 0.44 (n = 34)
Hemoglobin, g/dL (n)	14.26±1.10 (n = 34)
Urate, mg/dL (n)	4.1 ± 1.4 (n = 35)
Creatinine, mg/dL (n)	0.80 ± 0.17 (n = 35)
Ferritin, ng/mL (n)	139.3 ± 143.5 (n = 19)
Calcium, mg/dL (n)	9.5 ± 0.3 (n = 33)

Triglycerides, mg/dL (n)	87.2 ± 43.8 (n = 35)
Transferrin, mg/dL (n)	252.6 ± 41.3 (n = 18)
TSH (μIU/mL)	2.6 ±1.17 (n = 32)

714

715

716 **Supplementary Table S1.**

717 Longitudinal changes in clinical and biochemical parameters during the 12-month follow-
718 up. Values are presented as mean ± standard deviation (SD). Measurements were obtained
719 at baseline, 6 months, and 12 months. Sample size for each parameter is indicated in
720 parentheses and varies according to availability of routine laboratory testing.
721 Longitudinal changes represent descriptive comparisons across follow-up time points and
722 were not included as primary predictors in the multivariate models unless otherwise
723 specified in the main text. BMI: body mass index; EDSS: Expanded Disability Status
724 Scale; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TSH: thyroid-
725 stimulating hormone

726

	Baseline	6 months	12 months
BMI, kg/m² (n)	25.63 ± 5.26 (n = 40)	24.85 ± 4.90 (n = 38)	24.40 ± 4.56 (n = 31)
EDSS score	1.14 ± 1.58 (n = 40)	1.28 ± 1.64 (n = 40)	1.23 ± 1.71 (n = 38)
Plasma homocysteine, µmol/L (n)	10.91 ± 6.94 (n = 41)	8.24 ± 3.33 (n = 40)	8.27 ± 2.42 (n = 36)
Vitamin B12, pg/mL (n)	445.4 ± 179.51 (n = 10)	430.75 ± 123.47 (n = 4)	539.9 ± 115.24 (n = 10)
Folate, ng/mL (n)	7.59 ± 3.72 (n = 9)	9.08 ± 6.18 (n = 4)	8.01 ± 3.22 (n = 10)
Albumin, g/dL (n)	4.71 ± 0.30 (n = 34)	4.61 ± 0.28 (n = 39)	4.6 ± 0.23 (n = 32)
Total cholesterol, mg/dL (n)	189.17 ± 28.94 (n = 35)	189.91 ± 31.87 (n = 32)	195.04 ± 33.11 (n = 25)

LDL cholesterol, mg/dL (n)	114.83 ± 24.58 (n = 18)	107.79 ± 22.24 (n = 24)	108.41 ± 29.11 (n = 22)
HDL cholesterol, mg/dL (n)	62.17 ± 17.20 (n = 18)	59.54±14.60 (n = 24)	67.17 ± 13.45 (n = 23)
Vitamin D, ng/mL (n)	27.26 ± 8.86 (n = 24)	28.77±9.10 (n = 36)	27.12 ± 9.07 (n = 32)
Serum iron, µg/dL (n)	78.58 ± 24.89 (n = 19)	98.9±32.33 (n = 29)	87.17 ± 37.37 (n = 30)
Glucose, mg/dL (n)	81.77 ± 8.71 (n = 35)	84.23±9.08 (n = 39)	85.88 ± 11.59 (n = 33)
Total proteins, g/dL (n)	7.05 ± 0.44 (n = 34)	7.05±0.45 (n = 39)	7.01 ± 0.49 (n = 33)
Hemoglobin, g/dL (n)	14.26 ± 1.10 (n = 34)	14.17±1.20 (n = 39)	14.26 ± 1.16 (n = 32)
Urate, mg/dL (n)	4.14 ± 1.47 (n = 35)	4.21±1.06 (n = 39)	4.02 ± 1.05 (n = 33)
Creatinine, mg/dL (n)	0.80 ± 0.17 (n = 35)	0.79±0.14 (n = 39)	0.80 ± 0.14 (n = 33)
Ferritin, ng/mL (n)	139.26 ± 143.47 (n = 19)	134.14±150.7 (n = 29)	118.13 ± 119.82 (n = 30)
Calcium, mg/dL (n)	9.48 ± 0.29 (n = 33)	9.42±0.35 (n = 38)	9.47 ± 0.42 (n = 33)
Triglycerides, mg/dL (n)	87.2 ± 43.79 (n = 35)	93.0±36.53 (n = 32)	91.92 ± 44.69 (n = 25)
Transferrin, mg/dL (n)	252.61 ± 41.27 (n = 18)	257.97±46.39 (n = 30)	255.53 ± 46.23 (n = 30)
TSH (µIU/mL)	2.60 ± 1.17 (n = 32)	3.13±2.15 (n = 38)	2.7 ± 1.47 (n = 33)

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728

729 **Supplementary Table S2.**

730 Dietary assessment protocol, food-frequency questionnaire, and adherence categorization

Component	Timing	Information collected or procedure	Use in the study
General clinical and lifestyle data	First nutrition visit	Date of birth, food allergies or intolerances, medication other than MS treatment, relevant comorbidities, work schedule, usual place of meals, and usual physical activity.	Used to personalize dietary counselling and adapt recommendations to the patient's clinical and lifestyle context.
24-hour dietary recall	First nutrition visit	Record of all foods and drinks consumed during the previous 24 hours, including approximate timing of meals.	Used to characterize baseline dietary habits and identify areas for improvement.
Food-frequency questionnaire	First nutrition visit	Weekly frequency of intake of main food groups, including legumes, pasta, rice, vegetables, meat, fish, and other relevant foods. Food preferences and usual cooking or preparation methods were also recorded.	Used as a pragmatic dietary assessment tool. For analysis, information was summarized into broad categories: vegetables/plant-based foods, lean non-processed protein sources, and nutritionally unfavourable foods.
Anthropometric assessment	First and follow-up visits	Weight, height, wrist circumference, body complexion, BMI, and estimated total energy expenditure.	Used to define weight-related objectives and guide whether the diet should be normocaloric or mildly hypocaloric.
Initial dietary guidance	First nutrition visit	Individualized preliminary guidance for the following week, including approximate meal timing, breakfast, mid-morning snack when applicable, afternoon snack when applicable, and general conditions for lunch and dinner.	Used to introduce the key dietary targets before prescribing the full weekly plan.
Core dietary targets	From first visit onward	Diet aimed to be normocaloric or mildly hypocaloric depending on baseline BMI, weight objective, and clinical context. It included all food groups unless restricted by allergies, intolerances, or strong aversions. Targets included increased intake of fish, especially oily fish, vegetables, including leafy vegetables and different cooking methods, and fruit.	Provided the common nutritional framework applied to all participants, although menus were individualized.
7-day dietary diary	Between first and second visit	Patients recorded all food intake during the following 7 days using a diary provided at the first visit.	Used to evaluate understanding of the initial guidance, assess real dietary habits, and design the first detailed weekly diet.
Second nutrition visit	Second visit	Review of the 7-day dietary diary, assessment of adherence to the initial guidance, review of preferences, and weight measurement.	Used to prescribe a detailed weekly dietary plan adapted to the patient's needs and preferences.
Weekly dietary plan	Second and subsequent visits	Menus were specified for each day. Energy, carbohydrate, and protein targets were based on the first-visit assessment and adjusted cautiously when needed. Household measures were used to explain approximate portion sizes rather than requiring patients to weigh food.	Used to provide practical individualized dietary counselling while maintaining the core study targets.
Foods to avoid or restrict	Second and subsequent visits	Patients were advised to avoid or consume only sporadically foods considered nutritionally unfavourable, including processed foods, sugar-rich foods, processed meats, sugar-sweetened drinks, alcoholic drinks, and high-calorie preparations.	Used to support the reduction of foods considered metabolically unfavourable.
Follow-up consultations	Subsequent visits	Interview-based assessment of adherence to the weekly plan, especially key dietary targets; weight monitoring; adaptation of the next weekly plan to work schedule, family routines, special meals, holidays, celebrations, and expected physical activity.	Used to maintain adherence, avoid monotony, and adapt the intervention to real-life circumstances.
Adherence categorization	First and second semesters	Adherence was rated as poor, irregular, or good based on follow-up interviews, review of compliance with the prescribed plan, and, when applicable, weight evolution.	Used to construct the semester adherence score: poor adherence = 1, irregular adherence = 2, good adherence = 3. The total adherence score was calculated as the sum of both semester scores, ranging from 2 to 6.
Interpretation of adherence score	12-month analysis	Higher scores indicated more sustained adherence to the dietary counselling programme.	The score was interpreted as a pragmatic clinical monitoring tool, not as a validated quantitative dietary assessment instrument. Analyses using this score were considered exploratory.

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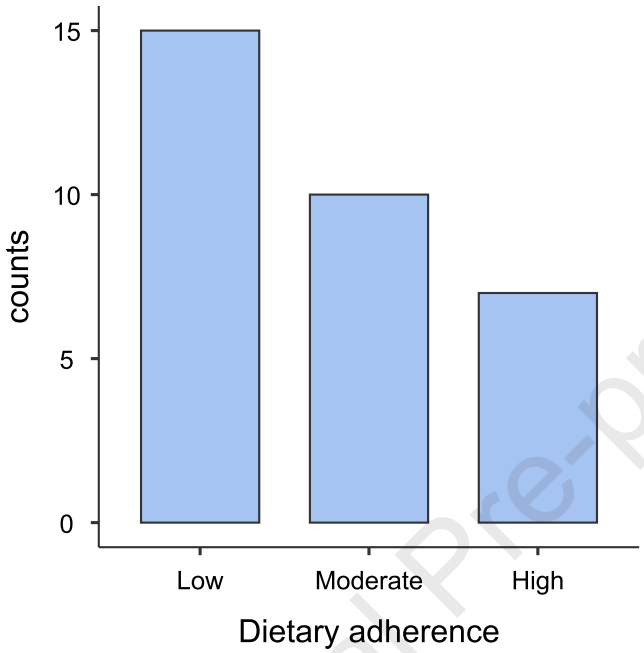

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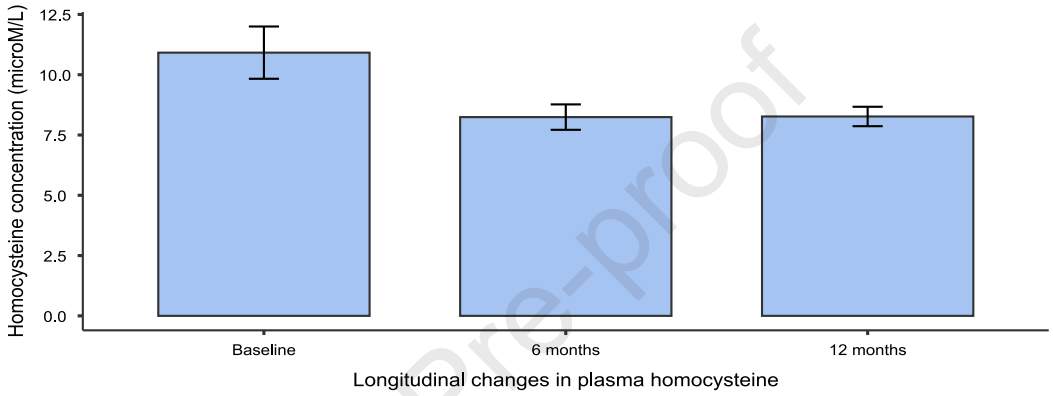

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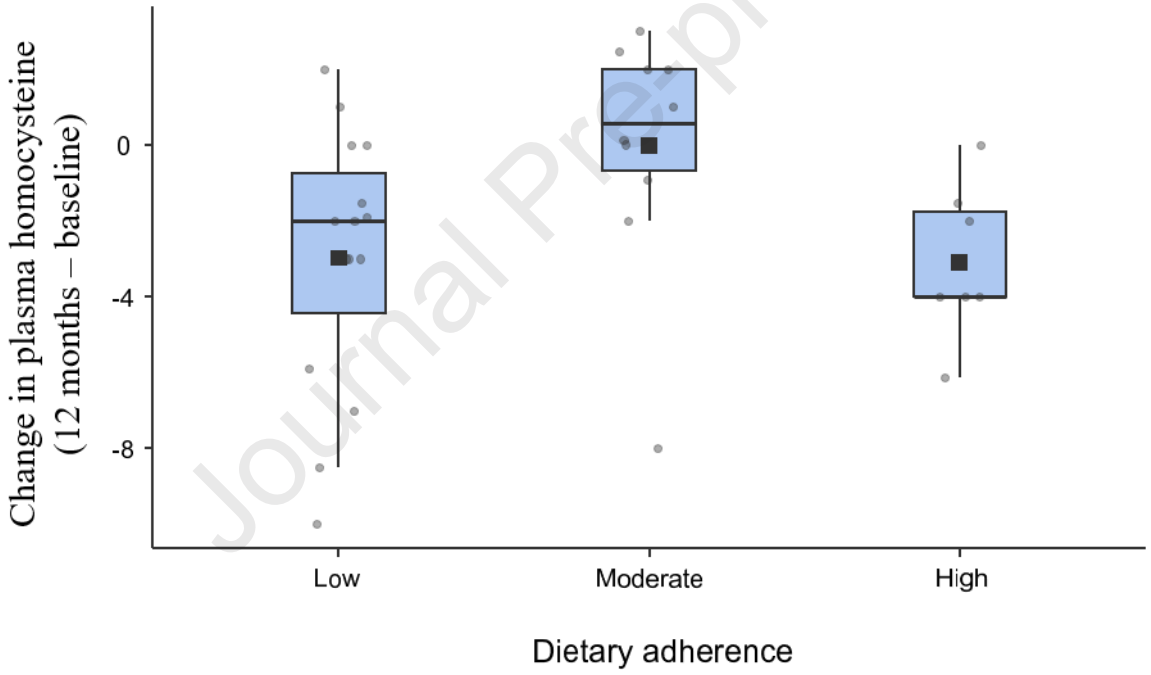

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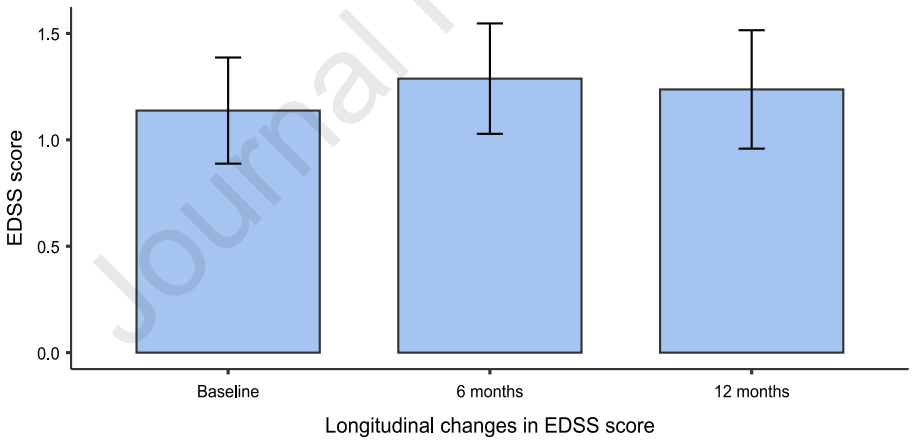

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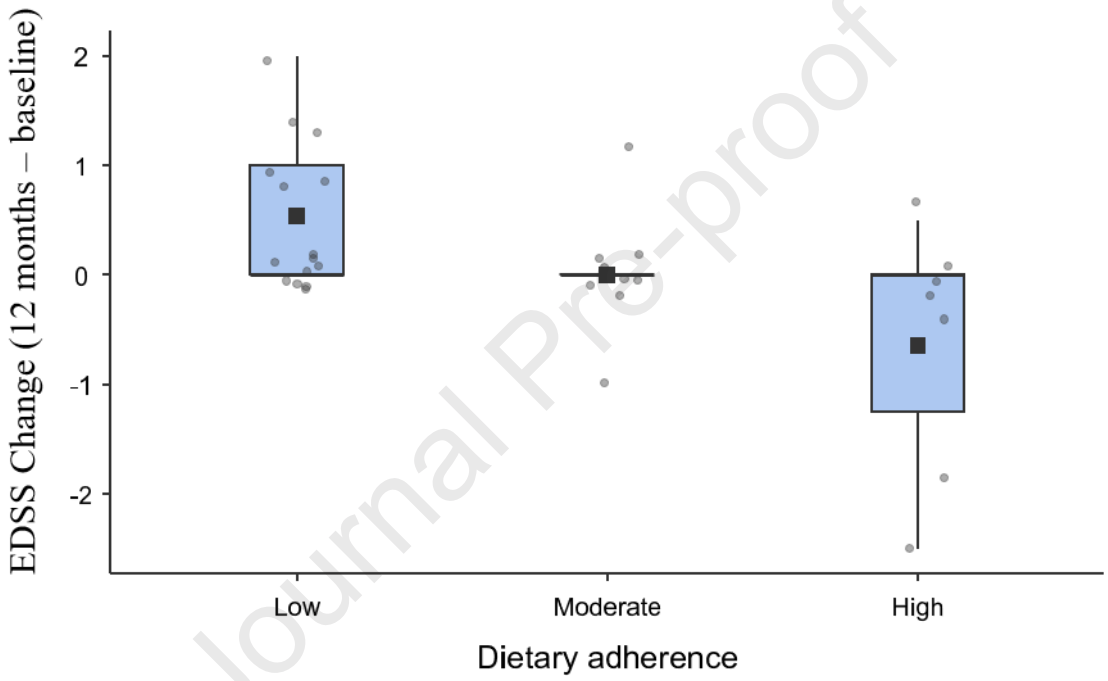

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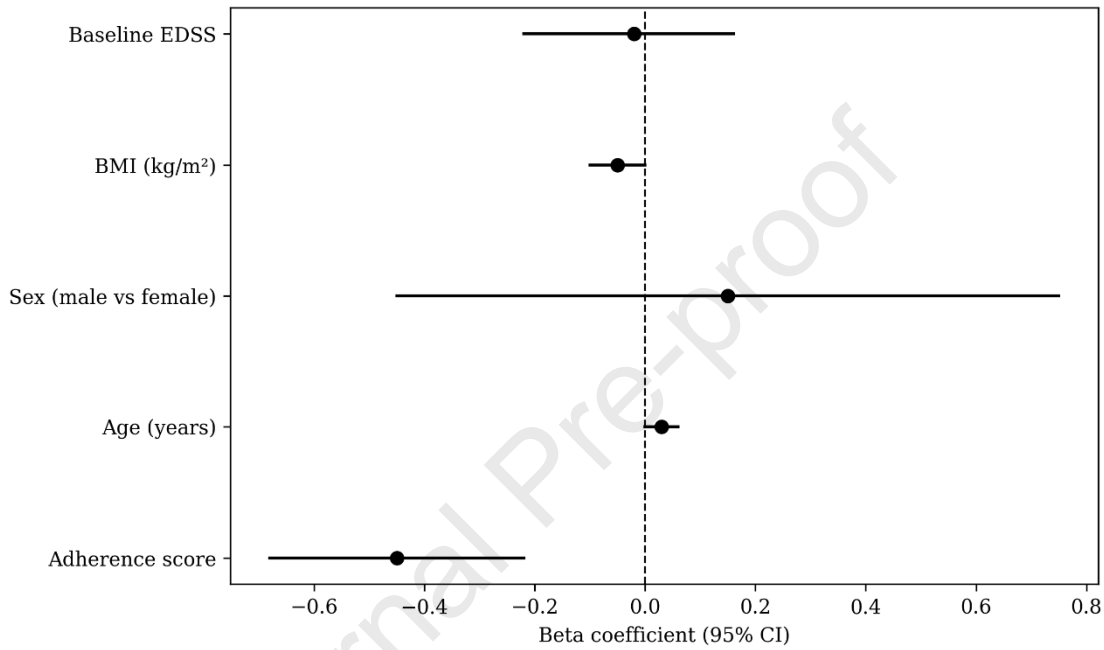

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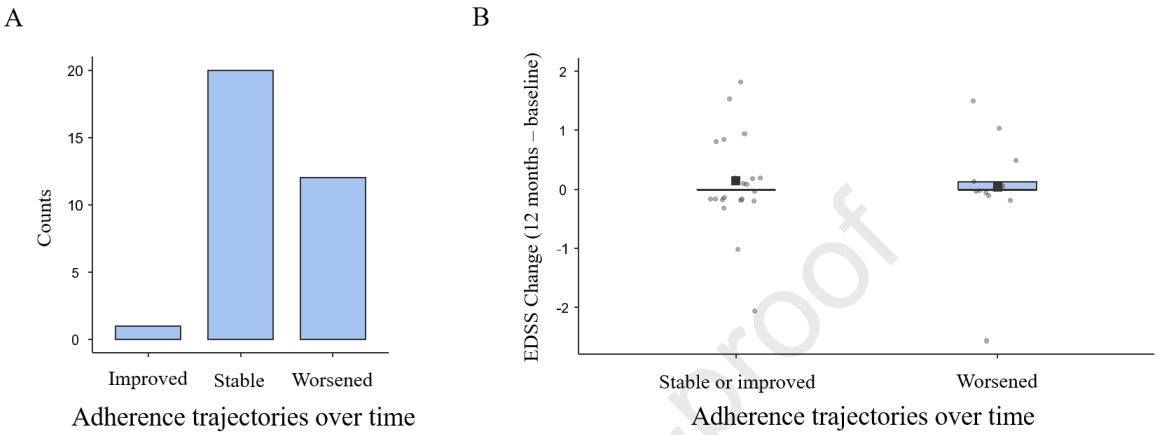

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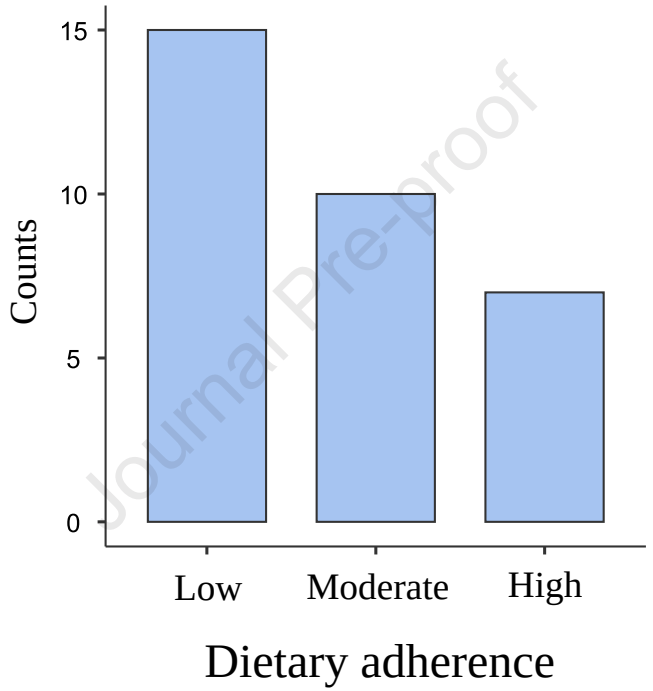
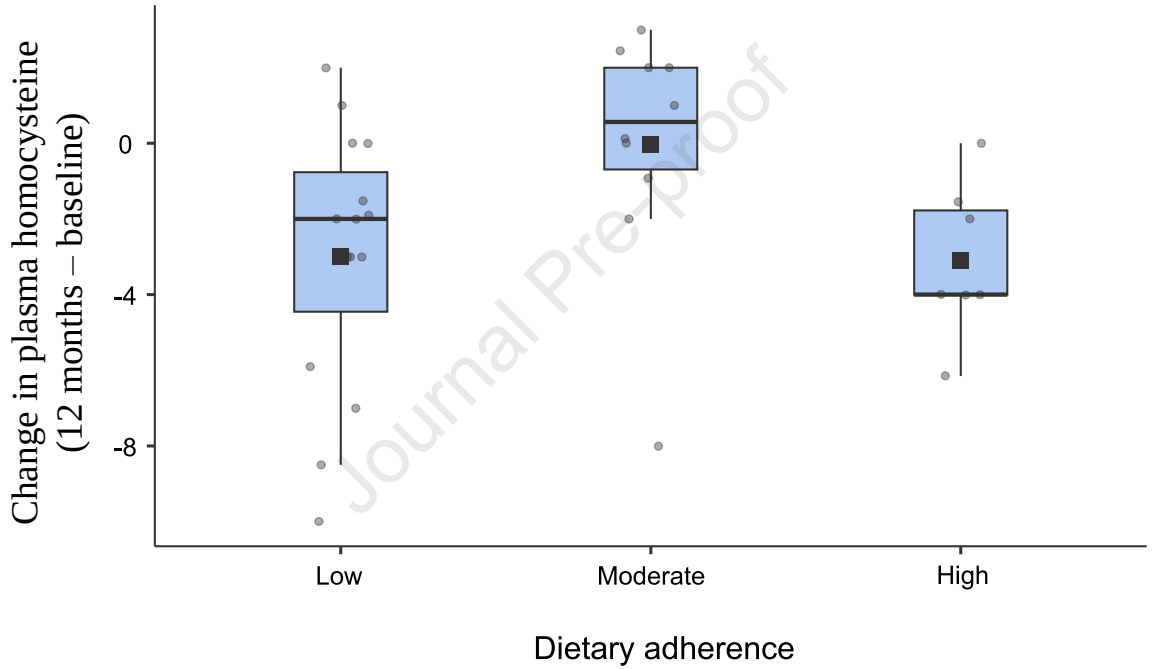
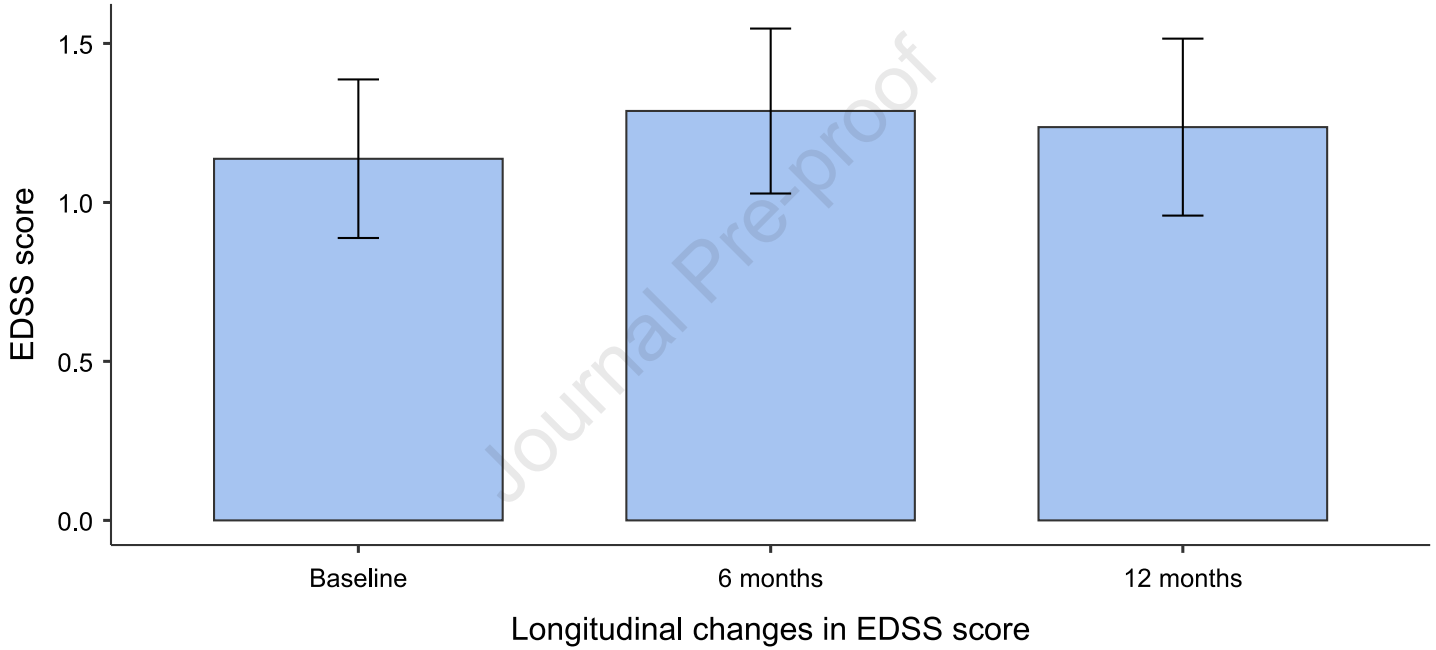
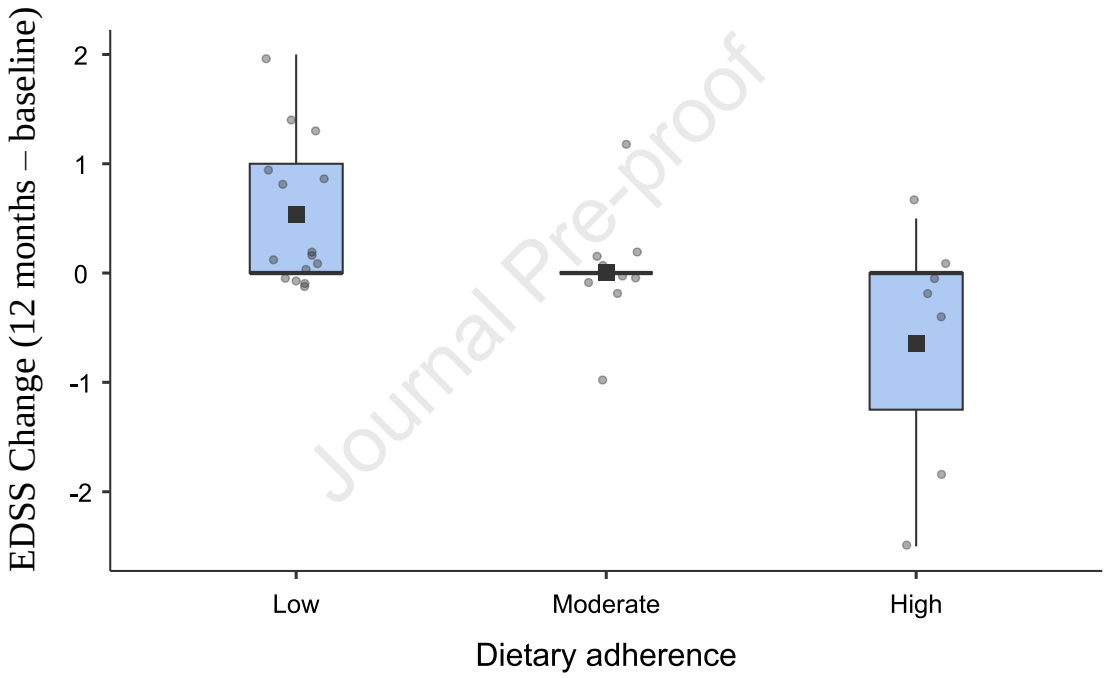
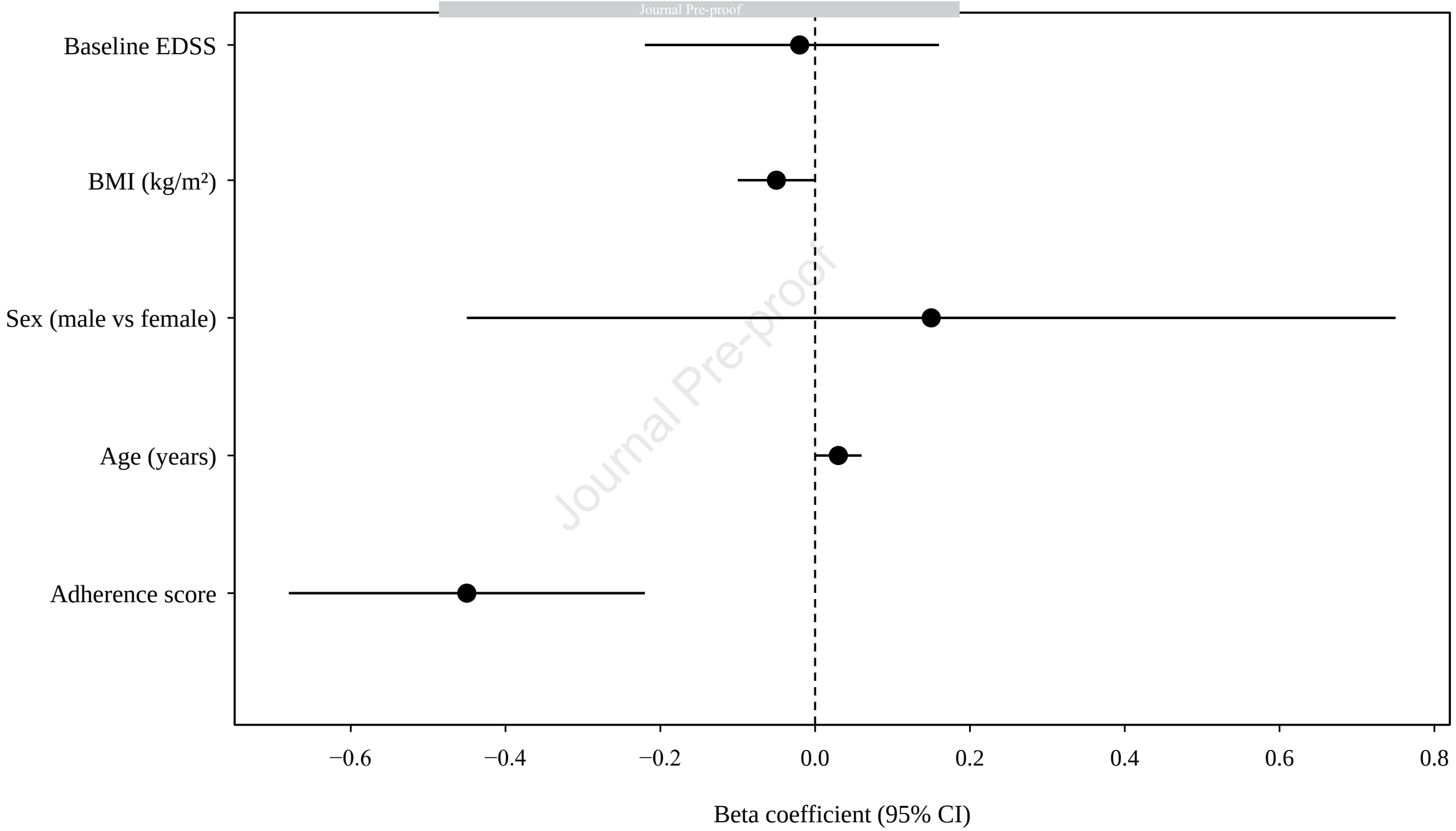


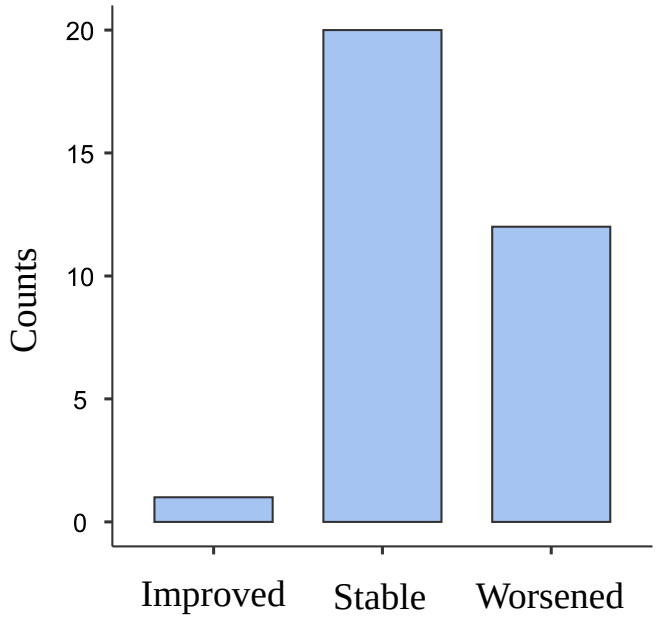






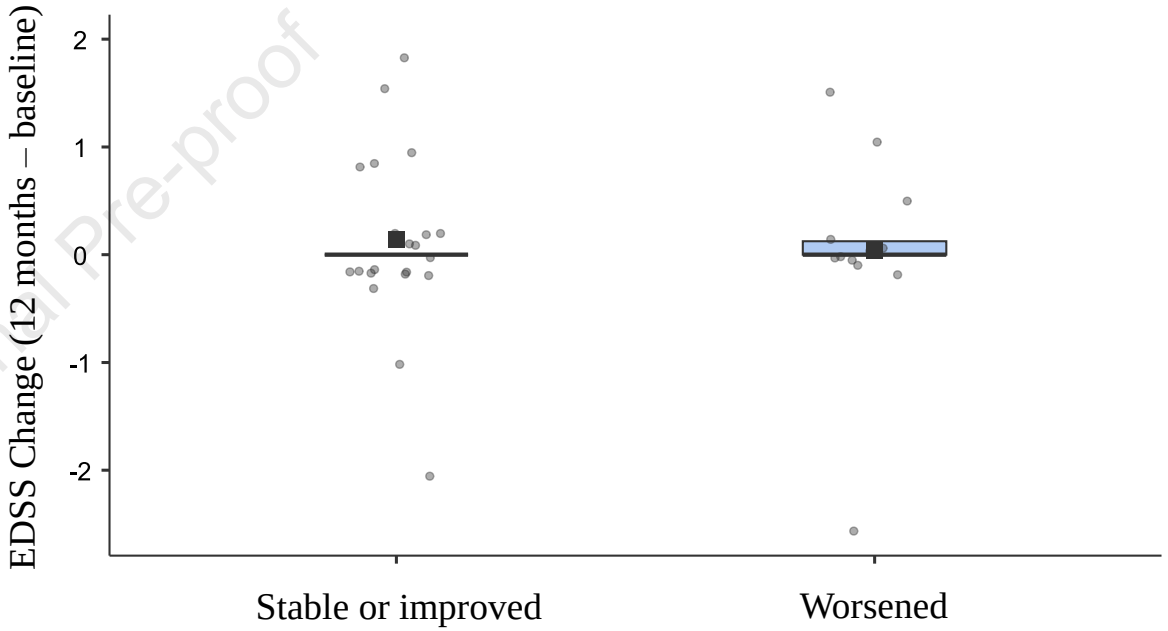


A



Adherence trajectories over time

B



Adherence trajectories over time