A Case Study on the Impact of Exercise and Essential Amino Acid Supplementation on Physical Fitness and Body Composition

Theocaris Ispoglou1, Panagiotis Ferentinos1, Konstantinos Prokopidis2, Cameron Blake1, Luke Aldrich1, Antonis Elia3, Matthew Lees4, and Karen Hind5

1Leeds Beckett University
2University of Liverpool
3Royal Institute of Technology School of Education and Communication in Engineering Science
4University of Toronto
5Wolfson Research Institute

April 25, 2023

Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disorder causing nerve sheath demyelination and symptoms such as muscle weakness, mobility decline, and lack of coordination1,2, generating unique health challenges and an economic burden3-5. Patients experience reductions in bone and skeletal muscle mass6, muscle strength and function7,8, and increased fracture risk9, negatively impacting quality of life10. Lower limb strength impairments8, poor balance11 and spasticity12 also contribute to a lower quality of life10. Pharmacological treatments have been the primary option for patients with MS13.

Recent research has explored non-pharmacological treatments such as exercise, nutritional supplementation, and improved sleep quality14-21. Resistance exercise (RE) is particularly beneficial for MS rehabilitation, as it improves muscle strength and function, mobility, quality of life22,23, and the immune system24. High-dose vitamin D supplementation raises interleukin-10 (IL-10) levels in MS patients19, who tend to have lower levels of IL-10, which may contribute to the disease’s development25. However, RE and vitamin D may not always improve physical fitness20,21. This could be due to unsatisfactory energy and protein intake, containing essential amino acids (EAA), which are necessary to stimulate muscle protein synthesis (MPS) and ultimately address sarcopenia26, a condition prevalent in MS patients27. EAA-based supplements enriched with L-leucine increase protein intake and optimise MPS in healthy older adults without compromising total energy intake during mealtimes28,29 and plasma EAA concentration is associated with muscle function in older women in the community30. A higher protein intake, including specific amino acids, may positively impact bone health through mechanisms such as increasing insulin-like growth factor 1 (IGF-1)31,32. Therefore, addressing dietary protein deficiencies alongside RE may optimise musculoskeletal health and function in female MS patients. This case study evaluated the effects of a 24-week home-based intervention, including EAAs and vitamin D3 supplementation, on muscle, bone, muscle strength, and function in a female patient with MS.

Materials and Methods

Study Design and Ethics Approval

The study utilised a case study design. The patient completed three testing sessions separated by 12 weeks,
including measures of strength, body composition, functional performance tests, and blood tests for selected physiological variables. The study adhered to the Helsinki Declaration of 1964 and received institutional ethics approval. The study was part of a larger trial registered in a Clinical Trial's Registry with identification number ISRCTN12419961.

**Participant Information**

A 57-year-old female with MS, identified as frail and at risk of malnutrition, participated in the study. Baseline scores were 5 (symptomatic of sarcopenia) using the SARC-F questionnaire, 3 (frail) using the FRAIL scale, and 10 (at risk of malnutrition) using the mini-nutritional assessment questionnaire. The participant’s key characteristics are provided in Table 1.

**Preliminary screening and anthropometry**

The participant arrived at the laboratory between 07:00 and 09:00 in a fasted, euhydrated state, with no alcohol or exercise for 24 hours prior. Baseline stature, body mass, and blood pressure were recorded using standard equipment. Self-reported physical activity levels at baseline were estimated using the short-form IPAQ and showed low activity.

**Experimental protocol**

The participant completed a 24-week home-based exercise programme, doing upper (hand grip, wall press, seated dumbbell curls and seated reverse wrist curls) and lower body exercises (mini squats (assisted with a chair), calf raises, hip marching and a one leg stand), twice a week. The intervention promoted progressive overload and increased repetitions and sets over time. The exercise technique was based on NHS Choices (https://www.nhs.uk/live-well/exercise/). Rest periods of approximately 1-2 minutes were taken between sets. See Figure 1 for an outline of the intervention.

**Nutritional Supplementation**

The participant consumed 65ml gel-based supplements (GEL) twice daily with breakfast and lunch, providing 113.6 kcal, 21.9 g of carbohydrate, 7.5 g protein in the form of EAA, and 500IU (12.5 μg) of cholecalciferol (Vitrition UK Ltd.). The decision to supplement with EAA gel was based on suboptimal protein intakes in these meals. Compliance was monitored by counting unconsumed sachets. Table 2 shows the EAA profile of the GEL.

**Dietary Intake Assessment & Analysis**

The participant completed a 3-day self-report diet diary on three occasions (0, 10, and 22 weeks), which were analysed using Nutritics software (Nutritics, 2022).

**Body Composition Assessment**

Dual energy x-ray absorptiometry (DXA) (GE Lunar iDXA, GE Healthcare, Madison, WI) was used to assess total fat mass (TFM), lean tissue mass (LTM), bone mineral content (BMC) and total body fat percentage (TBF%), at weeks 0, 12, and 24 as described by Lees et al.

**Fitness Testing**

Tests included a handheld grip strength test (HGS), 30s arm curl (30ACT), 30s chair-stand tests (30CST), gait speed (GS) over 6m, and a six-minute minute walk test (6MWT). Ratings of perceived exertion (RPE) were recorded using the Borg Scale. These tests informed the training exercise protocol, as previously described.

**Blood sampling**

Blood samples were obtained from an antecubital vein. The samples were centrifuged, stored at -80°C, and later analysed for 25(OH)D3 and IGF-1 using commercially available ELISA kits, and amino acid content.

**LC-MS Sample and Standard Preparation**
Plasma samples were deproteinized by adding 1 ml of a 50:50 methanol:acetonitrile v/v solution containing 0.5% formic acid to 250 μl of plasma, using a dilution factor of 5. The resulting solution was vortexed for 30 seconds, then centrifuged and filtered before being stored in suitable glass vials. A labelled intermediate standard was added to ensure accurate quantification, while unlabelled standards were used to identify target analytes and optimise the chromatography and mass spectrometer acquisition parameters. Quality control samples were used to measure recovery and ensure correct method application.

**LC-MS Method and Analysis**

An ultra-high-pressure liquid chromatography (UHPLC) analytical apparatus, the Nexera X2 module (Shimadzu Corporation Europa GmbH, Milton Keynes, UK) with two pumps, an autosampler, column oven, and column switching valve was used. Chromatographic separation of compounds occurred using a Raptor Polar X column (Restek, Bellefonte, USA). Mass spectrometric detection was carried out by the LCMS-8045 triple quadrupole instrument. The analysis was carried out in positive MRM mode with a single transition for each compound. Mobile phases used were as follows: A – water, 0.5% formic acid; B – 9:1 Acetonitrile:Water v/v, 20Mm Ammonium Formate, formic acid (pH 3.0). A gradient elution was used according to a previously verified method (Restek, 2021): Gradient (%B) 0 min – 88%; 3.5 min – 88%; 8 min – 30%; 8.01 min – 88%; 10 min – 88%; flow rate 0.5ml/min, column over temperature 30°C. Samples and standards injected at 5μl injection volume and performed by an autosampler.

**Statistical Analysis**

Lab-Solutions Insight and Microsoft Excel were used for data handling and quantification. Results are presented as absolute values or means (+SD) of three days, when appropriate. Least significance change (LSC) was estimated by multiplying 2.77 with %CV precision errors for GE Lunar iDXA. Figure 1 was created with BioRender.Com.

**Results**

**Energy and Macronutrient Intake**

Compliance to the nutritional regime was 100%. Energy and relative protein intake averaged 1703(±348.2), 1360(±101.9) and 1364(±141.3)kcal/d and 1.04(±0.3), 1.32(±0.1) and 1.26(±0.1)g/kg/d at baseline, weeks 10 and 22, respectively. Table 3 provides additional details on the participant’s diet.

**Body Composition**

LTM and FM measures showed a greater percentage change in the initial 12 weeks and remained elevated at week 24 compared to baseline. BMI slightly increased, while TBF% and BMC remained relatively unchanged. See Table 4 for full details on body composition.

**Fitness Testing and Ratings of Perceived Exertion (RPE)**

Fitness tests showed significant improvement over the 24-week intervention, with a reduction in RPE in most tests (Table 5). The 6MWT showed the greatest improvement at week 24, with a distance covered over 100% longer than baseline, and a large decrease in RPE.

**Blood Variables**

Circulating [25(OH)D3] increased significantly from baseline (23.2 ng/mL) to 24 weeks (41.3 ng/mL), with a percentage change of 78%. IGF-1 showed a smaller increase of 6.9% over the same period (baseline=131.6 ng/mL; 24 weeks=140.7 ng/mL). The plasma concentration of 17 amino acids increased by 10.3% and 19.1% at week 12 and 24, respectively, compared to baseline (see Table 6).

**Discussion**

In this 24-week case study, a home-based intervention consisting of RE twice a week and EAA and vitamin D-enriched nutritional supplements twice daily improved body composition, muscle strength, physical performance, plasma amino acid profile, 25(OH)D3 concentration, and IGF-1 in a 57-year-old female with
MS. These findings suggest that exercise and nutritional interventions may improve the physical capacity of individuals with MS.

The 6-month intervention resulted in notable enhancements in performance tests, particularly in the 6MWT, which displayed a 125.6% increase at week 24 compared with baseline. Performance tests also showed decreased RPE scores, suggesting favourable physiological changes, with the most significant reduction observed after the 6MWT, an indirect measure of aerobic fitness. Despite not reaching the normal range for the 6MWT (498-604m), we can speculate that true physiological changes in cardiovascular fitness occurred, as RPE and heart rate are highly related, even though we did not calculate the heart rate walking speed index. The participant’s ACT scores were initially below normal and improved by week 24, while hand grip strength was within the normal range at baseline. The right arm HGS improved more than the left at weeks 12 and 24. The participant’s performance in the 30s CST was below the expected range, only achieving scores within the range at week 24. Gait speed, initially identified as sarcopenic (0.8m/s) at baseline, improved throughout the intervention. Body composition showed clinically significant increases in regional LTM (arms and legs) and FM, mainly due to increased FM in the legs. At 3 months, body mass increased but slightly decreased after mid-testing, possibly due to fear of weight gain. Week 10 and 22 dietary intakes suggest a slight caloric deficit, which may explain some changes in body composition.

Meta-analyses have shown significant improvements in timed up and go testing, short and long walk tests, and walking speed in MS patients following RE, supporting our findings. The addition of a sufficient per-meal EAA dose may have contributed to enhanced LTM and muscle strength. Our data suggest an increase in daily protein intake from 1 g/kg/d to 1.3 g/kg/d, with marked elevations in plasma NEAA (+18.3%) and EAA (+20.6%) at completion of the 24-week intervention due to 2xEAA gel supplementation. This aligns with previous work suggesting a relationship between plasma amino acids and muscle function in older women. The participant’s total sum of plasma amino acids increased, shifting away from the potential development of sarcopenia. The rise in alanine, arginine, glutamic acid, lysine, and IGF-1 supports the intervention’s potential to improve bone health, but larger studies are required to verify the role of amino acids in muscle protection.

Despite being included in our intervention, the effectiveness of vitamin D supplementation remains uncertain. While some studies suggest a positive effect of vitamin D and RE on strength, recent trials have yielded inconsistent results. Previous research has shown no changes in HGS and MS functional composite scores with weekly administration of vitamin D (20,000 IU) alone. High doses of vitamin D (50,000 IU every 5 days for 3 months) may promote anti-inflammatory properties by raising IL-10 levels, but the impact of lower doses, such as 20,000 IU/week, is controversial. Some studies report an inadequacy in suppressing markers of systemic inflammation, while others show increased TGF-β levels. Daily supplementation of 1000 IU cholecalciferol led to increased 25(OH)D3 concentration in our case, likely due to additive effect of supplementation and sunlight exposure from March to August.

Conclusions

In conclusion, this 6-month case study of a female with MS demonstrated the positive impact of incorporating resistance exercise and nutritional supplementation on body composition, muscle strength, and physical performance. However, because this was a case study, it precludes generalisation of outcomes, and further identical interventions with larger MS populations are needed to confirm their effectiveness. Despite this limitation, the intervention appears to be a safe and effective strategy for improving physical capacity in individuals with MS.

Author Contributions: TI: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualisation, writing original draft, writing, review and editing; PF: data curation, formal analysis, project administration, writing, review and editing; KP: data curation, visualisation, writing, review and editing; CB: data curation, formal analysis, software, writing original draft; LA: data curation, formal analysis, methodology, software, writing, review and editing; AE: data curation, formal analysis, methodology, software, writing, review and
Financial Disclosures: No conflict of interest has been reported.

Funding/Support: Institutional funding was granted by Leeds Beckett University.

Reference List


52. Nabuco HC, Tomeleri CM, Junior PS, et al. Effects of higher habitual protein intake on resistance-
training-induced changes in body composition and muscular strength in untrained older women: a clinical

53. Antoniak AE, Greig CA. The effect of combined resistance exercise training and vitamin D3 supplemen-
tation on musculoskeletal health and function in older adults: a systematic review and meta-analysis. *BMJ
open*. 2017;7(7):e014619.

54. Mohmen KS, Hammarstrom D, Pedersen K, et al. Vitamin D3 supplementation does not enhance the

55. Ashtari F, Toghianifar N, Zarkesh-Esfahani SH, Mansourian M. Short-term effect of high-dose vitamin
D on the level of interleukin 10 in patients with multiple sclerosis: a randomized, double-blind, placebo-

56. Bhargava P, Fitzgerald KC, Calabresi PA, Mowry EM. Metabolic alterations in multiple sclerosis and

57. Amirinejad R, Shirvani-Farsani Z, Gargari BN, Sahraian MA, Soltani BM, Behmanesh M. Vitamin D

58. Rosjo E, Steffensen LH, Jorgensen L, et al. Vitamin D supplementation and systemic inflammation in

59. Aivo J, Hanninen A, Ilonen J, Soili-Hanninen M. Vitamin D3 administration to MS patients leads
to increased serum levels of latency activated peptide (LAP) of TGF-beta. *Journal of neuroimmunol-

Hosted file

*figure 1.docx* available at https://authorea.com/users/611081/articles/639765-a-case-study-
on-the-impact-of-exercise-and-essential-amino-acid-supplementation-on-physical-fitness-
and-body-composition

Hosted file

*Table 1 Participant characteristics.docx* available at https://authorea.com/users/611081/
articles/639765-a-case-study-on-the-impact-of-exercise-and-essential-amino-acid-
supplementation-on-physical-fitness-and-body-composition

Hosted file

*Table 2.docx* available at https://authorea.com/users/611081/articles/639765-a-case-study-on-
the-impact-of-exercise-and-essential-amino-acid-supplementation-on-physical-fitness-and-
body-composition

Hosted file

*Table 3.docx* available at https://authorea.com/users/611081/articles/639765-a-case-study-on-
the-impact-of-exercise-and-essential-amino-acid-supplementation-on-physical-fitness-and-
body-composition

Hosted file

*Table 4.docx* available at https://authorea.com/users/611081/articles/639765-a-case-study-on-
the-impact-of-exercise-and-essential-amino-acid-supplementation-on-physical-fitness-and-
body-composition

Hosted file

Hosted file