



Associations Between Gut Microbiota, Cardiorespiratory Fitness, and Body Composition in Physically Active Women with Multiple Sclerosis: A Cross-Sectional Study

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SUMMARY/ABSTRACT

Starting point: Multiple sclerosis (MS) is associated with physiological impairments and gut microbiota dysbiosis. Whether physical activity mitigates these microbial shifts and how gut microbiota relates to cardiorespiratory fitness (CRF) in MS individuals remains unclear.

Group: Sixteen women with MS and sixteen healthy women meeting the WHO physical activity recommendations were included.

Methods: Gut microbiota composition was assessed by 16S rRNA gene sequencing targeting the V3–V4 region, body composition by bioimpedance analysis, and cardiorespiratory fitness by an incremental bicycle ergometer test.

Results: Despite being physically active, significantly lower CRF (e.g., VO_{2max}/kg , $Loadmax/kg$) and less favorable body composition (e.g., body fat percentage, waist circumference) were observed in MS women compared with healthy controls. Furthermore, reduced gut microbial diversity (Chao1) and lower relative abundance of several health-associated taxa (e.g., *Faecalibacterium*, *Coprococcus*, and *Prevotellaceae*) were identified in MS women. Notably, CRF emerged as the strongest discriminative factor between groups and was positively associated with microbial diversity and the abundance of health-associated bacterial taxa. Among the identified taxa, *Veillonella* demonstrated the strongest association with CRF, whereas *Coprococcus* was primarily associated with body composition.

Conclusions: Physically active MS women still differed from healthy controls in both physiological and microbial characteristics. CRF represented the strongest discriminative factor between groups and was positively associated with gut microbial diversity and health-associated bacterial taxa. Together, these findings suggest that CRF may represent an important component of the relationship between host physiology and gut microbiota composition in MS.

KEYWORDS

physical exercise, gut microbiota, *Veillonella*, *Coprococcus*, body fat

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1 INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system associated with progressive neurological dysfunction and reduced functional capacity ¹. Individuals with MS typically demonstrate lower levels of physical activity, largely due to fatigue, mobility limitations, and disease-related disability ². Consequently, reduced cardiorespiratory fitness (CRF) ³ and unfavorable body composition are commonly observed in MS ^{4,5}. Moreover, several studies have reported gut dysbiosis in individuals with MS compared with healthy controls ⁶. The gut microbiome plays a central role in immune homeostasis ⁷ and may contribute to the pathophysiology of neurological diseases, including MS ⁸. Reduced abundance of *Prevotella*, *Faecalibacterium prausnitzii*, *Bacteroides coprophilus*, and *Bacteroides fragilis* has been repeatedly observed in individuals with MS ^{9,10}.

Besides the positive effects of physical activity on cardiorespiratory fitness ^{11,12} and other physiological and biological processes impaired in MS ^{13,14}, growing evidence suggests that exercise may also modulate gut microbiota composition ¹⁵. Exercise has been associated with improved microbial diversity, higher abundance of butyrate-producing bacteria, and better balance between beneficial and pathogenic bacterial communities in neurodegenerative diseases ^{16,17}. In addition, previous studies demonstrated associations

between CRF and gut microbiota composition, whereas lower aerobic fitness was linked to pro-inflammatory bacteria^{18,19}.

Although lifetime physical activity has been related to brain health and gut bacterial diversity in MS²⁰, no study to date has specifically examined the relationship between CRF and gut microbiota composition in physically active individuals with MS. Therefore, the aim of this cross-sectional study was to compare physically active MS women and physically active healthy controls. In addition, we investigated whether the gut microbiota dysbiosis previously reported in individuals with MS is also observed in physically active women with MS and how gut microbiota composition is associated with cardiorespiratory fitness. We hypothesized that physically active MS women would exhibit a less pronounced alterations in gut microbiota composition than previously reported in sedentary MS populations. We further hypothesized that higher cardiorespiratory fitness would be associated with greater microbial diversity and a more favorable abundance of anti-inflammatory bacterial taxa.

2 GROUP AND METHODS

2.1 RECRUITMENT AND STUDY SUBJECTS

The patients with relapsing-remitting MS, diagnosed according to official diagnostic criteria²¹, were recruited from the Multiple Sclerosis Center at the Second Department of Neurology, Comenius University and University Hospital Bratislava, Slovakia. Data for this study were obtained in a clinical trial named Gut Microbiota Composition, Cognitive Function, and Physical Fitness in Multiple Sclerosis Patients (ClinicalTrials.gov, No: NCT06220409). This study was approved by the Ethics Committee of Derer's University Hospital in Bratislava with No. 27/2022 and was carried out in accordance with the Helsinki Declaration Principles for Human Experiments. After reading the written informed consent and after an explanation of the study steps and a discussion with the investigators, signed informed consent was obtained from all subjects prior to their participation in the study.

This cross-sectional study involved 16 physically active MS women (MS) and 16 physically active healthy women (CTRL). MS women had been treated mostly with second-line disease-modifying therapy (DMT) (fingolimod, natalizumab, ocrelizumab, and cladribine). Inclusion criteria were: (a) age 30–60 years and (b) achievement of at least 150 minutes of moderate-intensity physical activity per week according to the World Health Organization (WHO) recommendations²². Compliance with the WHO physical activity recommendations was assessed using a self-reported questionnaire. Exclusion criteria included (a) recent relapse less than 3 months (also any worsening with the need for corticosteroids); (b) serious comorbidities, including psychiatric diseases; (c) use of antibiotics for a period of 2 weeks or supplementary probiotics during the last 2 months; and (d) pregnancy or breastfeeding.

2.2 BODY COMPOSITION ASSESSMENT

The examination was carried at the Faculty of Physical Education and Sports Comenius University in Bratislava. A personal and family medical history was taken. Anthropometric parameters and body composition was measured using InBody 230 (Serial, USB, LookingBody Basic 120), including muscular mass, visceral fat, body weight, body height, and body fat percentage. BMI was computed as follows: weight in kilograms divided by height in meters squared. The waist and hip circumference were measured using a flexible tape.

2.3 CARDIORESPIRATORY FITNESS ASSESSMENT

Cardiorespiratory fitness was measured using an incremental test on a bicycle ergometer (PowerCube, Ganshorn, Germany). Standardized climatic conditions were maintained, with a temperature of 20°C and a relative humidity of 50–60%. After a brief familiarization phase, the warm-up comprised five minutes of cycling at 0.5 w.kg⁻¹ power output, increasing the load by 0.25 w.kg⁻¹ per minute until voluntary exhaustion. The last step should have been held for an entire minute to record the maximum workload. The aim was to achieve maximum effort in 8 to 12 minutes²³. Maximum exhaustion was confirmed when at least one of the two objective criteria were met: HR_{max} > 220 minus age or RER peak > 1.10²⁴. A medical doctor and an exercise physiologist supervised the operations during the testing. The measurements for the final ten seconds of every exercise were averaged.

2.4 GUT MICROBIOTA ASSESSMENT

Total DNA from fecal samples was extracted with the ZymoBIOMICS DNA/RNA kit (Zymo Research, Irvine, CA, United States) according to the manufacturer's protocol. NGS libraries were prepared with the 16S Microbiome NGS Assay (ViennaLab Diagnostics GmbH, Vienna, Austria). In the first PCR step, locus-specific primers amplified the highly variable V3-V4 regions. A second low-cycle PCR was performed during data demultiplexing, incorporating dual index sequences to allocate reads to individual samples. Both PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). DNA library quantification was conducted using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), while DNA profiles were verified with an Agilent 2100 Bioanalyzer and a high-sensitivity DNA kit (Agilent Technologies, Santa Clara, CA, USA). The DNA libraries were diluted to 2 nM, pooled in equimolar concentrations, and sequenced on the Illumina Nexseq 2000 platform (Illumina Inc., San Diego, CA, USA) using 300-bp paired-end reads.

Sequencing data were processed using the ViennaLab NGS Microbiome Assay software provided with the kit. Read preprocessing included merging, trimming, and quality control steps performed using BBMerge²⁵, Cutadapt²⁶, and SeqKit²⁷. Taxonomic classification at the species level was conducted using the CLARK sequence classification system²⁸, which relies on discriminative k-mers for sequence assignment. The reference database for classification was constructed from the SILVA²⁹ and UNITE³⁰ databases, while taxonomic annotations were obtained from the NCBI taxonomy database (<https://www.ncbi.nlm.nih.gov/taxonomy/>). Diversity metrics were subsequently calculated from species-level abundance data using MOTHUR³¹.

2.5 STATISTICAL ANALYSIS

Statistical analyses were performed with R software (v. 4.5.0)³². Prior to statistical analyses, microbial taxa present in fewer than 20% of samples were excluded. Data normality was assessed using the Shapiro–Wilk test. Group differences in mean values were analyzed using Student's t tests or Mann-Whitney U tests, depending on data normality. Effect sizes were calculated using Cohen's d for parametric comparisons and the rank-biserial correlation (r_{rb}) for non-parametric pairwise comparisons. Significant group differences in the cross-sectional study were visualized using violin plots. Beta diversity was calculated using Bray–Curtis dissimilarity and differences in microbiota composition between groups were tested using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Variable importance analysis was performed using a Random Forest algorithm implemented in the randomForestSRC package to identify the microbial, anthropometric, and cardiorespiratory fitness variables contributing most strongly to group discrimination. The importance of individual variables was evaluated using variable importance (VIMP), and partial dependence plots were generated to visualize the marginal effect of the highest-ranking variables. Model performance was internally assessed using the out-of-bag (OOB) samples generated during Random Forest training. The Spearman or Pearson correlation coefficient was used to analyze the correlations between the gut microbiota, cardiorespiratory fitness parameters, and anthropometric measurements, across the entire study cohort. Significant correlations were visualized cardiorespiratory correlation network graphs. All values are expressed as the mean $\pm$ standard deviation (SD) unless otherwise stated. The significance level of all statistical analyses was set at 0.05. P-values are reported as unadjusted p-values.

3 RESULTS

3.1 BODY COMPOSITION

Data from a total of 32 volunteers were analyzed, including physically active MS women and 16 physically active healthy women. Women with MS had a mean disease duration of 9.5 ± 6.3 years and a mean Expanded Disability Status Scale (EDSS) score of 3.7 ± 1.0 . Significant between-group differences were identified in body weight, body fat percentage, waist circumference, and waist-to-hip ratio (WHR), whereas the physically active MS women demonstrated significantly higher values compared to physically active healthy women (Table 1).

Table 1 – Between-group differences in body composition between physically active MS women and physically active healthy women.

	MS women (n = 16)	Healthy women (n = 16)	<i>p</i> -value	<i>r</i> (<i>rb</i>)
Body weight (kg)	75.26 ± 15.98	62.98 ± 11.41	0.012	0.523
Body height (cm)	168.53 ± 5.06	167.69 ± 4.64	0.571	0.101
BMI (kg/m²)	26.57 ± 5.71	22.28 ± 3.16	0.054	0.340
Body fat (%)	34.85 ± 10.03	20.85 ± 6.91	0.001	0.616
Muscle mass (kg)	26.21 ± 2.80	27.41 ± 3.66	0.396	0.150
Waist circumference (cm)	84.69 ± 13.29	72.06 ± 8.50	0.002	0.544
WHR	0.93 ± 0.10	0.83 ± 0.06	0.002	0.555

Values are presented as mean ± standard deviation. Table summarizing significant differences (bold) at $p < 0.05$ using the Mann-Whitney U test. Abbreviations: MS multiple sclerosis; BMI body mass index; WHR waist-to-hip ratio; *r*(*rb*) rank-biserial correlation.

3.2 CARDIORESPIRATORY FITNESS

Cardiorespiratory fitness was assessed using an incremental test on a bicycle ergometer. Significant between-group differences were identified in absolute VO₂max ($p < 0.001$, *r*(*rb*) = 0.945) and absolute Loadmax ($p < 0.001$, *r*(*rb*) = 0.992), as well as in VO₂max/kg ($p < 0.001$, *r*(*rb*) = 0.806), Loadmax/kg ($p < 0.001$, *r*(*rb*) = 0.840), and minute ventilation (VE, $p < 0.001$, *r*(*rb*) = 0.753). Physically active MS women demonstrated significantly lower values (absolute VO₂max 1.75 ± 0.33 l/min; absolute Loadmax 130.8 ± 22.4 W; VO₂max/kg: 24.14 ± 6.01; Loadmax/kg: 1.79 ± 0.41; VE: 63.65 ± 16.42) compared to physically active healthy women (absolute VO₂max 2.50 ± 0.27 l/min; absolute Loadmax 212.3 ± 22.7 W; VO₂max/kg: 40.99 ± 6.38; Loadmax/kg: 3.44 ± 0.57; VE: 92.96 ± 12.55) (Figure 1).

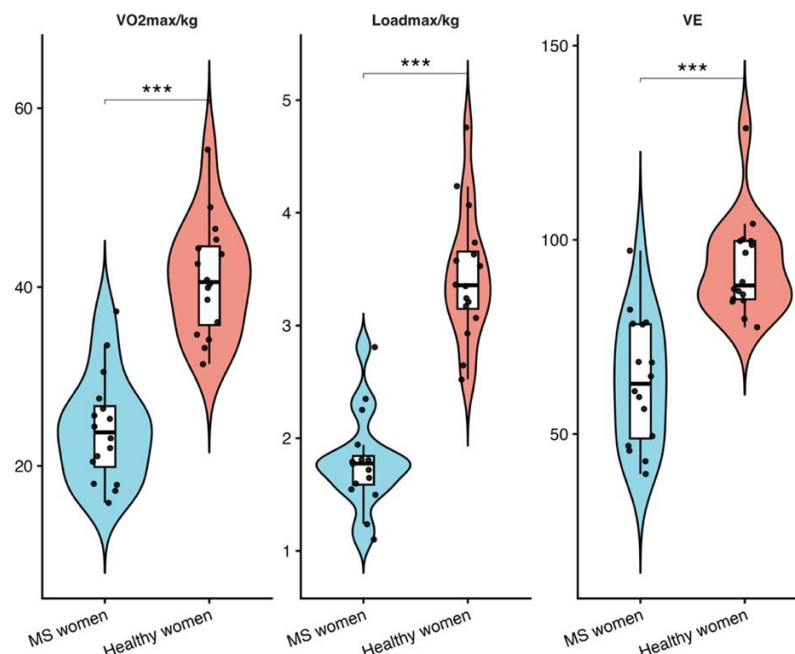


Figure 1 – Between-group differences in cardiorespiratory fitness between physically active MS women and physically active healthy women. Abbreviations: MS multiple sclerosis; VO₂max/kg weight-adjusted maximal oxygen uptake; Loadmax/kg weight-adjusted maximum load; VE Minute-ventilation; *** $p < 0.001$

3.3 GUT MICROBIOTA

Across all samples, the number of reads was 192.4 ± 61.3. Overall gut microbiota alpha diversity determined using the Chao1 index differed significantly between the groups ($p = 0.003$, *r*(*rb*) = 0.625),

whereas the physically active MS women demonstrated significantly lower Chao1 index (387.18 ± 90.47) compared to physically active healthy women (496.58 ± 81.58) (Figure 2). In contrast, β -diversity did not significantly differ between the groups across any taxonomic level (PERMANOVA, all $p > 0.05$).

The 16S rRNA gene method identified the gut microbiota's composition based on the relative abundance of microbes. A significant lower relative abundance of health-associated bacteria, including *Coprococcus* ($p = 0.008$, $r(\text{rb}) = 0.500$), *Faecalibacterium prausnitzii* ($p = 0.046$, $r(\text{rb}) = 0.353$), *Blautia massiliensis* ($p = 0.015$, $r(\text{rb}) = 0.504$), *Veillonella magna* ($p = 0.001$, $r(\text{rb}) = 0.641$), and *Prevotellaceae* ($p = 0.050$, $r(\text{rb}) = 0.406$) were identified in physically active MS women compared to physically active healthy women (Figure 2).

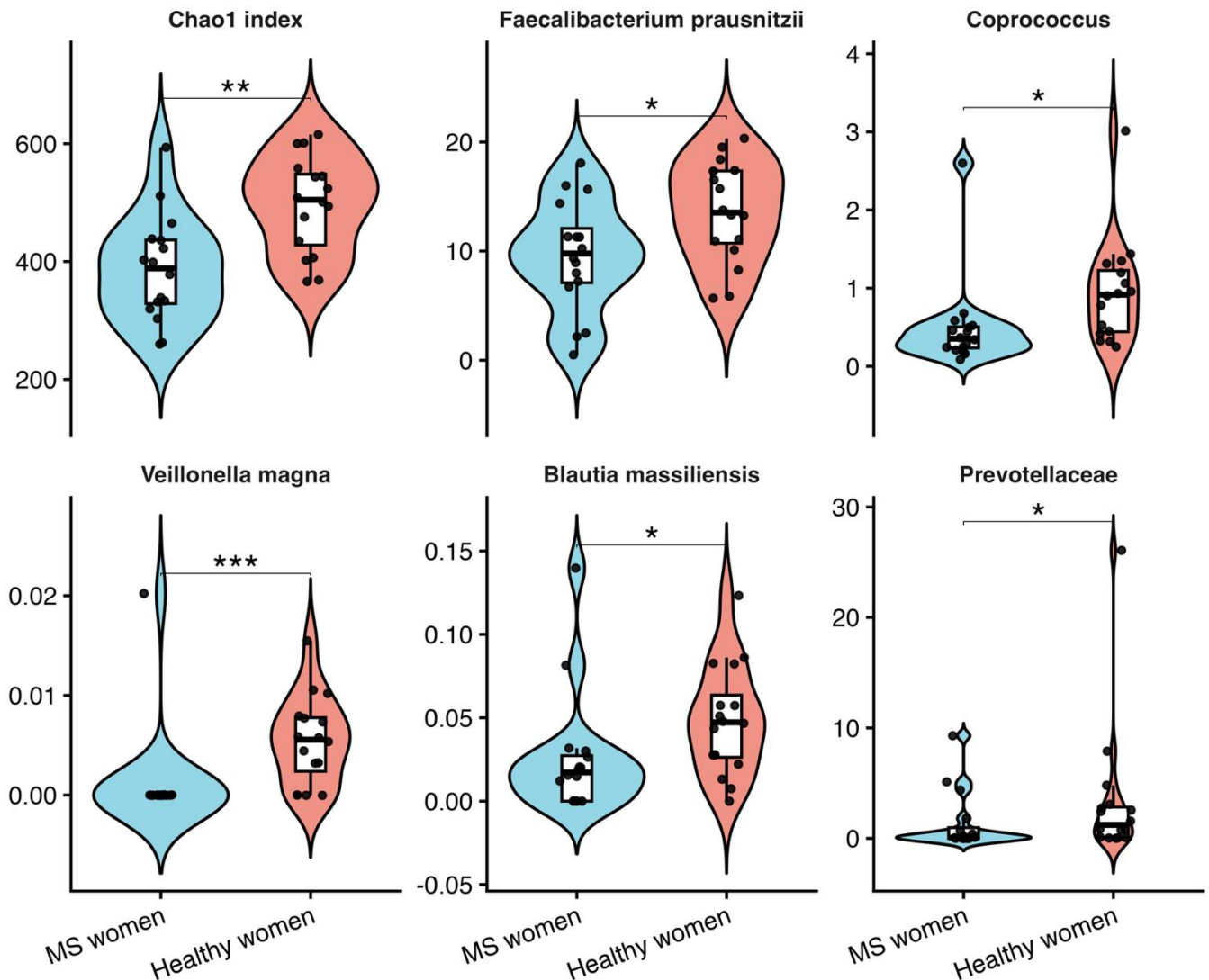


Figure 2 – Between-group differences in gut microbiota diversity (Chao1) and the relative abundance of health-related bacterial taxa between physically active MS women and physically active healthy women. Abbreviations: MS multiple sclerosis; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

3.4 RANDOM FOREST MACHINE LEARNING

Variable importance (VIMP) analysis revealed that cardiorespiratory fitness parameters, particularly $\text{VO}_{2\text{max}}$, $\text{VO}_{2\text{max}}/\text{kg}$, and $\text{Loadmax}/\text{kg}$, contributed most strongly to group discrimination across the overall cohort as well as within the individual groups. In contrast, body composition parameters exhibited relatively low discriminatory importance. Notably, several microbiota-related variables, including *Veillonella* spp. and *Coprococcus* spp., demonstrated comparable or even greater importance than selected body composition variables, particularly within the physically active MS women (Figure 3).

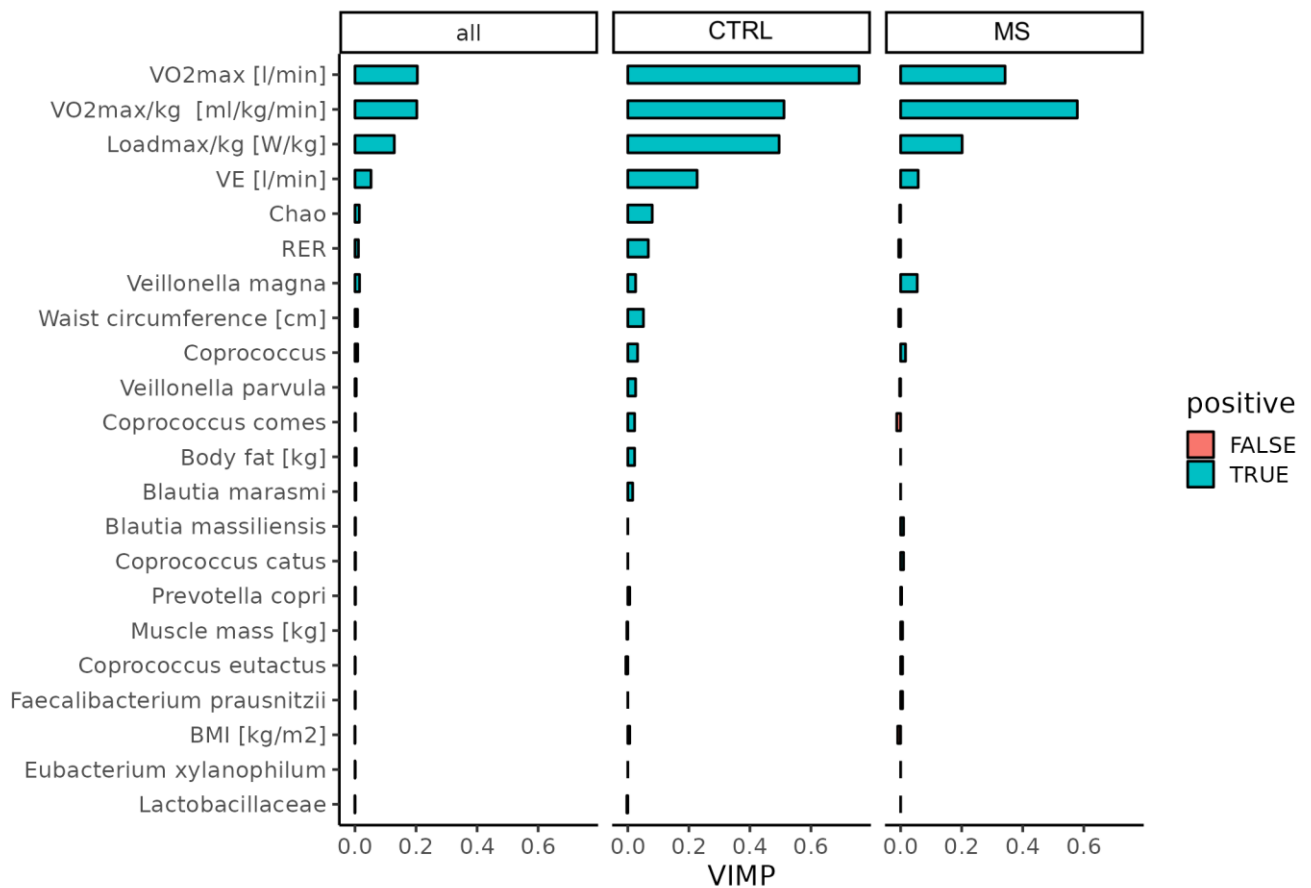


Figure 3 – Variable importance (VIMP) analysis derived from the Random Forest machine learning model evaluating the discriminatory contribution of gut microbiota taxa, body composition parameters, and cardiorespiratory fitness variables between physically active MS women and physically active healthy women. Panels represent the overall cohort (all) and analyses stratified by group (CTRL – physically active healthy women and MS – physically active MS women). Higher VIMP values indicate greater contribution of a variable to the predictive performance of the model. Abbreviations: MS multiple sclerosis.

3.5 GUT MICROBIOTA, CARDIORESPIRATORY FITNESS, AND BODY COMPOSITION ASSOCIATIONS

Spearman's correlation coefficient (ρ) was calculated to assess the relationships between significantly altered gut bacteria and significant changes in body composition and cardiorespiratory parameters (Figure 4). A significant positive association was observed between the Chao1 index, as well as relative abundance of health-associated bacteria (e.g., *Faecalibacterium prausnitzii*, *Lactobacillaceae*, *Enterococcus*, *Eubacterium xylanophilum*, *Veillonella magna*, *Veillonella rogosae*, *Phocaeicola massiliensis*, *Coprococcus* spp., *Prevotella rara*), with VO₂max/kg, Loadmax/kg, and VE. Conversely, the relative abundance of the aforementioned gut microbes was negatively associated with BMI, body fat percentage, and waist circumference.

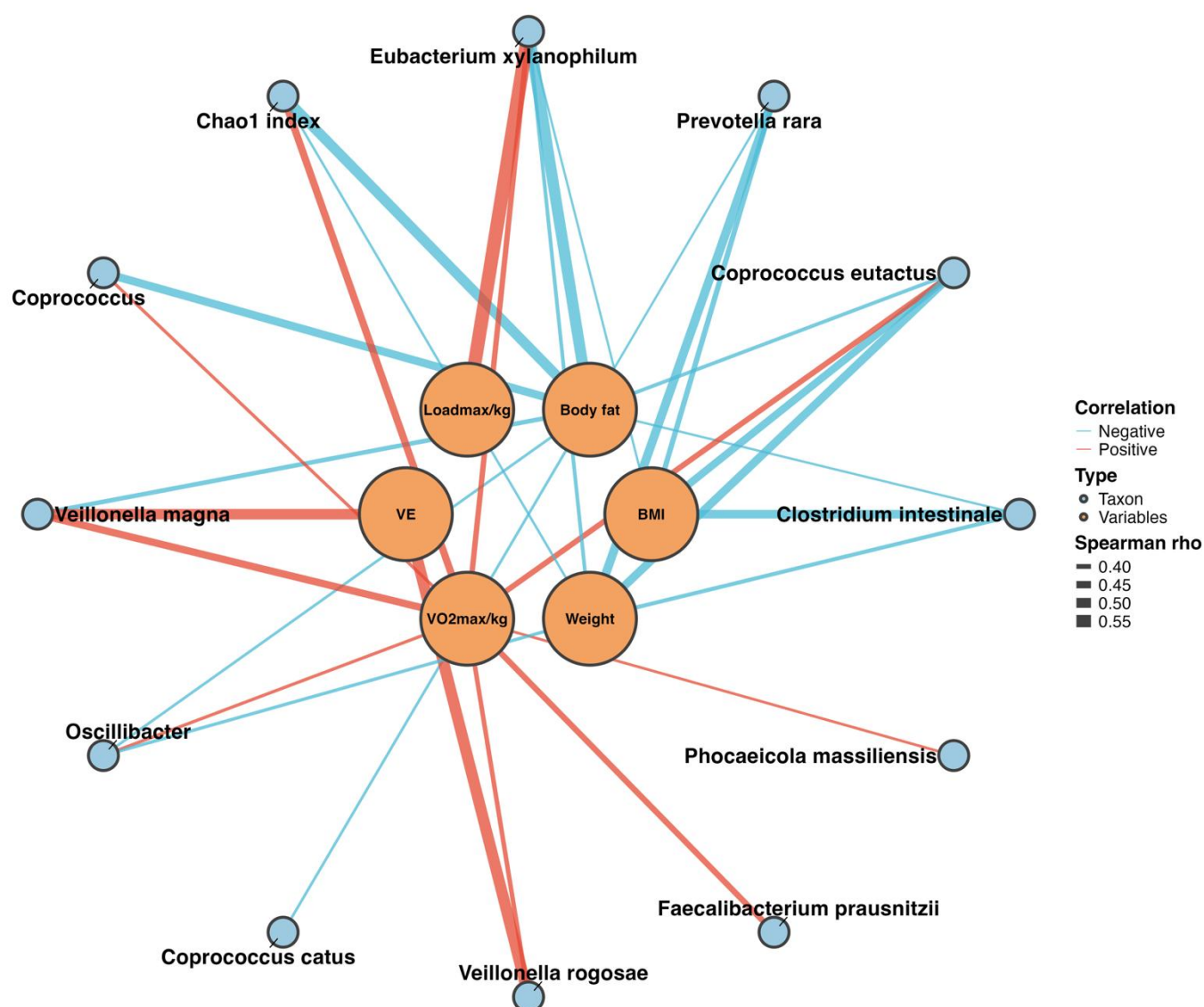


Figure 4 – Correlation network illustrating significant associations health-associated gut microbes, cardiorespiratory fitness and body composition characteristics. Nodes represent either microbiota taxa (blue nodes) or physiological variables (orange nodes). Edges represent statistically significant correlations ($p < 0.05$) identified using Spearman correlation analysis. Red edges indicate positive associations, whereas blue edges indicate negative associations. Edge thickness is proportional to the strength of the correlation coefficient (ρ). Abbreviations: VO2max/kg weight-adjusted maximal oxygen uptake; Loadmax/kg weight-adjusted maximum load; VE Minute-ventilation; BMI Body mass index; WC Waist circumference.

4 DISCUSSION

This cross-sectional study compared physically active women with multiple sclerosis (MS) and physically active healthy controls and further examined the associations between gut microbiota, cardiorespiratory fitness (CRF), and body composition. The main findings were as follows: (a) despite both groups meeting the World Health Organization (WHO) recommendations for physical activity, women with MS demonstrated lower CRF and less favorable body composition compared with healthy controls; b) CRF emerged as the strongest discriminative factor between physically active MS women and physically active healthy controls; and (c) the microbial profile partially reflected the physiological differences, including lower gut microbial alpha diversity and reduced relative abundance of several health-associated bacterial taxa in physically active MS women.

Obesity and unfavorable body composition are frequently reported in individuals with MS. These changes are commonly attributed to lower physical activity levels, fatigue, and mobility limitations^{4,5}.

However, women with MS in our study demonstrated significantly higher body weight, body fat percentage, and waist circumference compared with healthy controls despite meeting the WHO recommendations for physical activity. Importantly, women with MS appear to be particularly susceptible to rapid weight gain both at diagnosis and later during the disease course³³. Therefore, despite meeting the WHO physical activity recommendations, women with MS still exhibited less favorable body composition than healthy controls. Notably, although significant between-group differences in body composition were observed, these parameters demonstrated lower discriminative importance in the Random Forest model compared with CRF and specific health-associated gut bacteria.

Indeed, previous studies consistently demonstrated lower CRF in individuals with MS compared with healthy populations, while physical activity is considered an effective strategy for improving CRF^{3,11,12}. However, in our study, women with MS did not achieve CRF levels comparable to healthy controls despite being physically active. Importantly, several systems contributing to CRF appear to be impaired in individuals with MS, including skeletal muscle function³⁴, neuromuscular activation³⁵, mitochondrial oxidative capacity³⁶, and exercise tolerance³⁷. Moreover, previous study showed that some skeletal muscle oxidative adaptations remained unchanged even after exercise training in individuals with MS³⁷. Notably, CRF emerged as the strongest discriminative factor between physically active MS women and healthy controls, which was consistently supported by both Random Forest machine learning and correlation analyses. This finding is in line with accumulating evidence identifying CRF as an important predictor of overall physiological health, chronic disease risk, mortality, and neurodegenerative outcomes^{38,39}. Collectively, these observations highlight the potential of CRF to characterize the complex physiological alterations associated with MS.

Growing evidence further suggests that physiological and metabolic alterations are reflected in gut microbiota composition⁴⁰. Similarly, in our cohort, reduced alpha diversity and relative abundance of several health-associated taxa, including *Faecalibacterium*, *Coprococcus*, or *Prevotellaceae*, were observed in physically active MS women compared with healthy controls. These findings are consistent with previous reports describing reduced abundance of SCFA-producing and other beneficial bacteria in individuals with MS^{9,10}. Importantly, several significant associations between gut microbiota, CRF, and body composition were identified in our study. The gut microbiome diversity measured by Chao1 index was positively associated with CRF and negatively associated with adiposity-related measures. This finding is consistent with previous evidence linking higher cardiorespiratory fitness to higher gut microbial diversity^{19,41}, whereas lower microbial diversity has been associated with increased adiposity and adverse metabolic outcomes⁴².

Among the taxa associated with CRF in our study, genus *Veillonella* showed particularly strong relationships. Previous studies have linked *Veillonella* to aerobic performance through its ability to metabolize exercise-derived lactate and promote endurance capacity^{43,44}. Furthermore, in our cohort, body composition measures were negatively associated with abundance of health-associated taxa, particularly *Coprococcus* and other SCFA-producing bacteria. Reduced abundance of *Coprococcus* has previously been associated with obesity, metabolic dysfunction, and several neurological disorders^{45,46}. Moreover, SCFA-producing microorganisms contribute to metabolic homeostasis, gut barrier integrity, and immune regulation through the production of butyrate and other beneficial metabolites⁴⁷. Interestingly, in our study, both *Veillonella* and *Coprococcus* demonstrated greater discriminative importance in the Random Forest model than body composition parameters. Thus, these findings suggest that specific microbial taxa may serve as complementary markers of physiological and metabolic characteristics associated with MS beyond conventional anthropometric measures.

This study provides a unique perspective by focusing on physically active MS women and incorporating objective assessment of CRF rather than physical activity measures. Moreover, the relationship between gut microbiota composition and CRF remains largely unexplored in MS. However, several limitations should be acknowledged. First, the cross-sectional design limits the ability to determine the directionality or causality of the observed associations between CRF, body composition, and gut microbiota composition. Second, the relatively small sample size may have limited the statistical power to detect weaker associations. Furthermore, the Random Forest analysis should be interpreted as an exploratory approach

for ranking variables according to their contribution to group discrimination rather than as evidence of causal or predictive relationships. Third, the inclusion of inactive women with MS and inactive healthy controls would have allowed a more detailed evaluation of the relationship between physical activity, CRF, and gut microbiota composition. In addition, the heterogeneous use of disease-modifying therapies and differences in body composition between groups may have influenced the observed microbiota profiles. Finally, dietary intake was not assessed and therefore its potential influence on gut microbiota composition could not be accounted for. Nevertheless, the combined evaluation of CRF, body composition, and gut microbiota provides novel insight into the associations between physiological characteristics and gut microbiota composition in physically active MS women.

5 CONCLUSIONS

In conclusion, disease-associated physiological and microbial differences were still observed in physically active women with multiple sclerosis despite meeting the WHO physical activity recommendations. Nevertheless, cardiorespiratory fitness emerged as the strongest factor distinguishing women with multiple sclerosis from healthy controls and was positively associated with gut microbial diversity and the abundance of health-associated bacterial taxa. Together, these findings suggest that cardiorespiratory fitness may represent an important component of the relationship between host physiology and gut microbiota composition in multiple sclerosis. Future longitudinal and intervention studies are needed to clarify the direction and causality of these associations.

6 ZUSAMMENFASSUNG

Ausgangspunkt: Die Multiple Sklerose (MS) ist mit physiologischen Beeinträchtigungen und einer Dysbiose der Darmmikrobiota verbunden. Ob körperliche Aktivität diese mikrobiellen Veränderungen abschwächt und wie die Darmmikrobiota mit der kardiorespiratorischen Fitness (CRF) bei Personen mit MS zusammenhängt, bleibt unklar.

Gruppe: Sechzehn Frauen mit MS und sechzehn gesunde Frauen, die die Empfehlungen der WHO für körperliche Aktivität erfüllten, wurden eingeschlossen.

Methoden: Die Zusammensetzung der Darmmikrobiota wurde mittels 16S-rRNA-Gensequenzierung unter Verwendung der V3–V4-Region untersucht, die Körperzusammensetzung mittels Bioimpedanzanalyse bestimmt und die kardiorespiratorische Fitness durch einen stufenweise ansteigenden Fahrradergometer test bewertet.

Ergebnisse: Trotz körperlicher Aktivität wurden bei Frauen mit MS im Vergleich zu gesunden Kontrollen eine signifikant niedrigere CRF (z. B. $\text{VO}_2\text{max/kg}$, Loadmax/kg) sowie eine weniger günstige Körperzusammensetzung (z. B. Körperfettanteil, Taillenumfang) beobachtet. Darüber hinaus wurden eine verringerte Diversität der Darmmikrobiota (Chao1) und eine geringere relative Häufigkeit mehrerer gesundheitsassoziiierter Taxa (z. B. *Faecalibacterium*, *Coprococcus* und *Prevotellaceae*) bei Frauen mit MS festgestellt. Bemerkenswerterweise erwies sich die CRF als der stärkste diskriminierende Faktor zwischen den Gruppen und war positiv mit der mikrobiellen Diversität sowie der Häufigkeit gesundheitsassoziiierter bakterieller Taxa verbunden. Unter den identifizierten Taxa zeigte *Veillonella* die stärkste Assoziation mit der CRF, während *Coprococcus* hauptsächlich mit der Körperzusammensetzung assoziiert war.

Schlussfolgerungen: Körperlich aktive Frauen mit MS unterschieden sich weiterhin von gesunden Kontrollen sowohl hinsichtlich physiologischer als auch mikrobieller Merkmale. Die CRF stellte den stärksten diskriminierenden Faktor zwischen den Gruppen dar und war positiv mit der Diversität der Darmmikrobiota sowie gesundheitsassoziierten bakteriellen Taxa verbunden. Zusammengefasst deuten diese Ergebnisse darauf hin, dass die kardiorespiratorische Fitness eine wichtige Komponente der Beziehung zwischen der Physiologie des Wirts und der Zusammensetzung der Darmmikrobiota bei Multipler Sklerose darstellen könnte.

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8 DECLARATION OF INTEREST STATEMENT

The authors declare no conflict of interest.

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10 AUTHORS' CONTRIBUTIONS

Libuša Nechalová: Writing – original draft, Investigation, Formal analysis, Data curation, Funding acquisition. **Ivan Hric:** Writing – review and editing, Formal analysis, Visualization, Data curation. **Marián Grendár:** Data curation, Visualization. **Darina Slezáková:** Investigation, Conducted surveys, Interpretation of results, Funding acquisition. **Michal Minár:** Investigation, Conducted surveys, Interpretation of results, Funding acquisition. **Viktor Bielik:** Writing – review and editing, Resources, Methodology, Funding acquisition, Conceptualization.

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