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External Exposome of Italian adults with Multiple Sclerosis focusing on nutrition-related inflammation: results from the cross-sectional EXPOSITION study

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Abstract

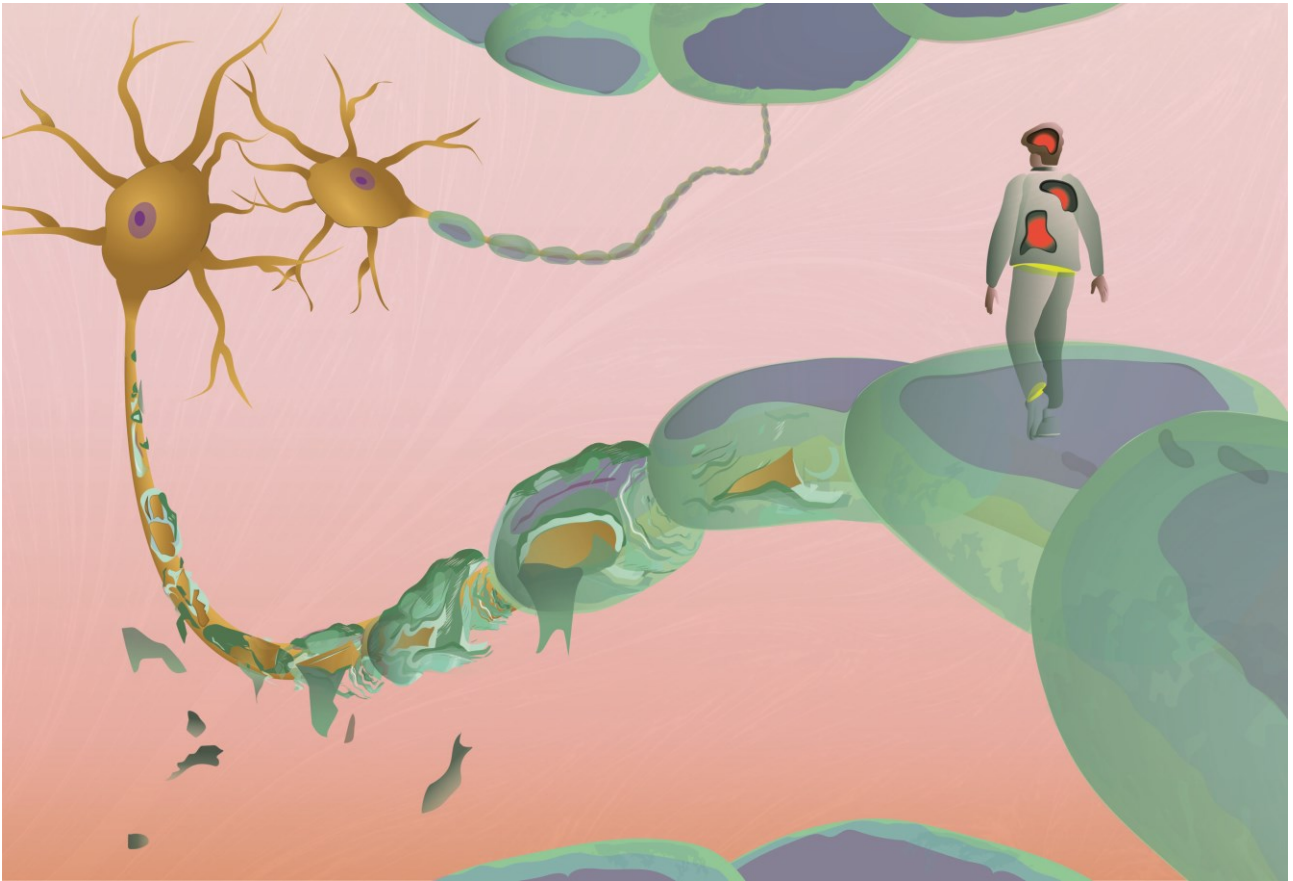
Background: The cause of multiple sclerosis (MS) is unknown and likely multifactorial, including genetic, environmental and lifestyle factors. Some dietary patterns have been studied in relation to MS, however the data are scarce, especially about the role of nutrients, dietary inflammation and body composition.

Aims: This thesis aims to fill these gaps by assessing nutrition and lifestyle habits in people with MS, examining sex-specific differences and their associations with phase angle and diet-related inflammation.

Methods: This thesis is part of the EXPOSITION cross-sectional study, which studied the exposome of people with MS living in the provinces of Milan and Pavia, Italy. Nutritional status, body composition, and lifestyle habits of people with MS (pwMS) were assessed through anthropometric measurements and bioelectrical impedance analysis (BIA) for phase angle, alongside validated questionnaires. Dietary intake was evaluated with a food frequency questionnaire and 24-hour recalls; dietary inflammatory potential with the Dietary Inflammatory Index (DII); and Mediterranean diet adherence with the MEDI-LITE score. Physical activity (IPAQ), sleep quality (PSQI), smoking status, and quality of life (MSQOL-29) were also recorded. Non-parametric tests were used for all variable comparisons, while LASSO regression was applied to explore associations and identify key predictors of phase angle and DII.

Results: In total, 131 pwMS (67% women, mean age 46 years) were included in this analysis, with low disability (median EDSS 1.5) and a median phase angle of 5.35°. Overall dietary patterns reflected a good adherence to the Mediterranean diet (median MEDI-LITE score 11), though the population reported a highly pro-inflammatory diet (median DII 4.1). Vitamin D insufficiency was common, with a median serum 25(OH)D of 22 ng/ml despite frequent supplement use, and sleep quality was generally poor, especially among women. Regression analyses showed that higher selenium intake and physical activity were associated with higher phase angle, whereas older age and female sex predicted lower values. Additionally, higher serum 25(OH)D was independently linked to lower DII scores, suggesting a role of vitamin D in modulating the inflammatory potential of the diet.

Conclusion: The results of this thesis support future personalized interventions combining anti-inflammatory diets, vitamin D levels optimization and tailored physical activity programs to improve muscle health, and consequently phase angle and overall well-being in pwMS. Longitudinal studies including participants with a wider range of disability are needed to confirm these effects and guide evidence-based strategies.



Chapter 1 Introduction

Definition, symptoms and types of MS

Multiple sclerosis (MS) is the most common neurological, inflammatory, autoimmune disorder affecting the central nervous system (CNS) that causes demyelination and neuronal injury in young adults (1). In particular, the immune system attacks the brain and spinal cord, causing inflammation and destroying the myelin sheath of the nerves. As a result, CNS plaques are created in the brain and spinal cord composed by inflammatory cells leading to interruptions to the correct nerve transmissions and consequently, into neurological-related symptoms and dysfunction (2).

Symptoms of MS can vary widely among persons and within the same person and be unpredictable, since the disease may affect different regions of the CNS. Some of the most common symptoms include: walking difficulties because of weakness, loss of balance, tremor and fatigue; sensory disturbances, such as tingling, numbness and itching; vision problems, such as blurred vision and diplopia; sexual problems; cognitive and emotional impairment, such as depression, memory problems and inability to learn; dizziness and vertigo and intestinal and urinary system problems (1,3). In high-income countries and in Europe, the highest percentage of people with MS (pwMS) (21%) are living with moderate disability according to the Expanded Disability Scale (4).

There are three types of MS. Globally, around 85% of pwMS are diagnosed with relapsing-remitting MS (RRMS), 12% with primary progressive MS (PPMS) and 3% remain with unknown type of MS (4). As RRMS is defined the type of MS that appears with recurrent attacks with total or partial recovery called relapses, while the inactive periods of the disease are called remissions. The period before a new relapse can fluctuate from weeks to years. On the other hand, PPMS is defined by the progressive worsening of the symptoms with no acute relapses as in RRMS. Secondary progressive MS (SPMS) is a phase of the disease that occurs usually after years of RRMS diagnosis and usually people with RRMS make the transition to SPMS (5). According to Rovaris et al (2006), the mean time to progressive phase from RRMS to SPMS is around 19 years (6). Moreover, regarding sex differences, males with MS have a worse prognosis compared to females by showing more difficult recovery from relapses, greater brain volume loss, cognitive impairment, disability development and transition to SPMS (7). On the other hand, women may experience more inflammatory activity and relapses and hormonal changes in women during reproductive phases such as pregnancy, breastfeeding and menopause, can have an impact on MS progression and activity (8).

According to Graves et al (2023), age is an important factor for the frequency of relapses. In particular, children are 3-5 more likely to experience relapses than adults, while young adults have more relapses than older ones (9). Additionally, the rate of disability accumulation is inversely associated to age, meaning that pwMS diagnosed earlier with the disease arrive to ambulatory disability slower than pwMS diagnosed at an older age (10).

MS epidemiology

According to the newest report of Atlas of MS 2020-2022 including data from 138 countries representing 87% of the world, MS prevalence is 2.8 million people living with MS worldwide compared to 2.3 million in 2013, meaning that one in 3,000 people lives with MS. The available data derives highly from high-income countries (94%), while only 37% of low-income countries provided data. Specifically, Africa is the WHO region with the lowest available evidence, covering only 56% of the countries. The prevalence of MS increases with increasing distance from the equator. The WHO Region with the highest prevalence of MS is Europe with 133 pwMS per 100,000 (4,11). On the other hand, migration studies have shown that moving from low- to high-risk countries during childhood decreases the risk of MS and vice versa (12,13).

Multiple sclerosis is a condition that occurs mostly in females (69% globally), while it varies based on the region. The reasons for this difference are unknown, but genetic, lifestyle, social and environmental factors may contribute to this incidence, so further research is fundamental in order to understand better the etiology of the disease and be able to prevent it based on the sex (4,11).

Multiple sclerosis is the most common cause of disability in young adults. The average age of MS diagnosis is around 32 years old globally, however the disease at pwMS with PPMS tends to appear at an older age at around 45 years old (14). Multiple sclerosis is less common in children and adolescents with at least 30,000 of them living with the condition (4).

Specifically, in Italy, based on the latest report of the Atlas of MS, the prevalence of MS is high, with around 133,000 people living with MS and 3,600 people being diagnosed each year. The average age of the diagnosis is 30 years old and around 90% of the diagnosis are usually RRMS (4).

MS etiology and the role of inflammation

As mentioned, MS is an inflammatory, autoimmune disease due to the development of immune system reactions against the CNS, resulting in neuroinflammation (15). The initial step of MS pathology is the invasion of autoreactive CD4+ T cells into the CNS, which start inflammatory reactions, for this reason CD4+ T cells are found in the lesions and CSF of pwMS (16). Even though MS was considered a T-cell mediated disease, recent research has demonstrated the importance of

B cells in MS pathology through antibody-dependent and -independent effects (15). B lymphocytes and their cytokines also produce lymphotoxin and TNF- α , which promote inflammation (17).

Still, the causes of MS are mostly unknown and they are attributed to environmental, genetic and epigenetic factors. For example, the heritability of MS within families provides evidence for the importance of genetic factors for the development of MS, while the Human Leukocyte Antigen (HLA) region of chromosome 6 has been associated with risk of MS, too, with carriers of the HLA DRB1*15:01 allele having around 3 times more chances of developing MS (18).

On the other hand, known environmental risk factors of MS include vitamin D deficiency due to low sunlight exposure (19), cigarette smoking (20), obesity in early life (21) and exposure to viral and bacterial agents such as Epstein Barr virus, causing infectious mononucleosis (22). The role of diet in the risk of MS is currently studied, giving some first indications about the dietary patterns, food groups and nutrients that may enhance or decrease the risk of MS, showing that diets that are proinflammatory may increase the risk of MS, however further research is necessary to come to clear conclusions (23).

Diagnosis of MS

The diagnosis of MS depends initially on neurological symptoms and signs (24). For the recognition of MS from other neurological diseases, the most common tool used for the diagnosis are the 2017McDonald criteria, which is based on clinical, imaging and fluid indicators (25). The determination of dissemination in space and time are important for the accurate diagnosis of MS by neurologists. Dissemination in space is described by the development of lesions in four anatomical positions in the CNS; for the diagnosis of MS, one or more T2-hyperintense lesions in at least two of these locations are required, while dissemination in time describes the creation of new lesions over time (26). Magnetic resonance imaging (MRI) is used for the diagnosis and standardized protocols have been suggested for baseline and follow-up, but also for brain and spinal cord imaging. Furthermore, a cerebrospinal fluid (CSF) analysis may take place according to the new criteria (13). McDonald 2017 criteria are based on the number of clinical attacks and the number of lesions found with objective clinical evidence, based on these finding, additional data may be needed for the diagnosis of MS according to the guidelines (26).

Treatment of MS

No cure is currently available for the elimination of MS. The strategies followed for the treatment of MS aim to shift the immune cell system from a pro-inflammatory to an anti-inflammatory phenotype through the regulation of T and B cells and anti-inflammatory macrophages (15). The

therapies followed by pwMS are disease-modifying therapies (DMTs) based on the symptoms and type of MS with the purpose to reduce the duration and frequency of acute relapses and provide symptoms relief (27). The majority of DMTs have anti-inflammatory effects with the goal of decreasing the relapse rate, MRI-based activity and disability worsening (25). Nowadays, only a drug, called ocrelizumab, is used for PPMS, which decreases the disability progression (28). Dalfampridine, on the other hand, is the first drug approved for any type of MS for the improvement of walking in pwMS, while studies have shown that it could increase up to 25% the walking speed of pwMS compared to placebo (29). Overall, the treatments and DMTs provided to pwMS are based on the drug availability insurance policies and healthcare systems, too (25). Evidence has shown that the administration of DMTs from the first demyelinating clinical event suggestive of MS may prolong the time till the next relapse, showing the importance of early diagnosis and initiation of MS management (30).

External exposome factors

Diet and MS

The role of diet in MS is still highly not investigated and the evidence provided is limited. Most of the studies focus on the role of diet on symptomatology, disability and disease outcome in pwMS, while data regarding associations between diet and risk of MS are scarce (31,32). The understanding of the role of diet is fundamental for the development of guidelines both for the prevention and the management of MS. Known dietary patterns have been studied in relation to MS, but mostly with few data and often with low sample size.

Diet, inflammation and MS

As mentioned before MS is an inflammatory disease and a proinflammatory diet could play a role on the onset of the disease and the disease progression. As proinflammatory foods are defined for example, alcoholic beverages (33), processed meat, refined sugar and carbohydrates, excessive red meat, fried food and sugar-sweetened beverages, while a diet that includes tea (34), coffee (35), a high consumption of fruit, vegetables, whole grains, plant-based proteins and healthy plant-derived fats, such as olive oil is considered anti-inflammatory (36). Flavonoids, such as anthocyanins, flavonols and flavanones, which can be found in different plant food such fruit, vegetables, grains roots are compounds that are known for their anti-inflammatory and anti-oxidative action, hence they are greatly used in nutraceuticals, pharmaceutical and other products (37). Regular consumption of flavonoids has been associated with reduced risk of neurodegenerative diseases, for this reason Solanski et al (2015), suggested flavonoid-based therapies for the early management of

these diseases (38). In spite of that, research about the potential preventive role of flavonoids on the risk and progression of MS are not available.

A good example of a pro-inflammatory diet predominant in multiple high-income countries, but mostly in USA, is the “Western diet”, which contributes to the incidence of multiple non-communicable diseases through chronic inflammation (39). A case-control study from Jordan using food frequency questionnaires (FFQs) using the Dietary Inflammatory Index (DII) for the evaluation of the overall inflammatory potential of diet showed that the more proinflammatory a diet is the higher the risk of MS and pwMS had significantly a more proinflammatory diet than controls, including high consumption of pro-inflammatory foods and nutrients (40). A case-control study from Iran studied the dietary habits of participants during adolescence and the risk of MS and found that a more pro-inflammatory diet during adolescence was associated with a higher risk of MS (41). In Australia, a case-control study using DII showed that a pro-inflammatory diet increases the risk of a first clinical diagnosis (FCD) of CNS demyelination in women, which is a predicting factor for MS (42). Another analysis, using data from the same Australian study and DII, showed that a pro-inflammatory diet increases the rate of relapses and fluid-attenuated inversion recovery (FLAIR) lesion volume in pwMS (43). Furthermore, an analysis using the same Australia study found that pro-inflammatory diet is associated with long-term depression and high anxiety levels in pwMS (44). Instead, a cross-sectional analysis in pwMS in Brazil showed that DII results did not differentiate the clinical outcomes of the participants (45).

Dietary patterns and MS

Mediterranean diet

The Mediterranean diet, one of the most known plant-based dietary patterns worldwide, which has been studied thoroughly for its benefits against multiple types of non-communicable diseases such as the cardiovascular diseases (46), has shown some positive results against MS, too. The Mediterranean diet is high in anti-inflammatory foods such as fruit, vegetable, whole grains and olive oil, while it includes a moderate amount of dairy and fish, a low consumption of red meat and a moderate consumption of red wine (47). A one-year cohort conducted in Italy showed that pwMS with lower MS severity scores had higher adherence to the Mediterranean diet, but only 31 pwMS were recruited (48), while a cross-sectional analysis also from Italy, found that higher adherence to the Mediterranean diet was associated with lower MS severity (49). Similar results were found in a cross-sectional Greek study, which found that higher adherence to the Mediterranean diet was associated with decreased disease progression, better quality of life (QoL) and higher muscle mass in pwMS (50). A randomized controlled trial in Iran, using as intervention a modified Mediterranean

diet and as control the traditional Iranian diet in pwMS, showed that the participants following the Mediterranean-style diet had improved inflammatory status and relapse rate (51). According to a systematic review by Abbasi et al (2023), higher adherence to a MD has been associated with lower fatigue and higher QoL in pwMS, however the uncertainty of evidence deriving from randomized-controlled trials was high (52).

Ketogenic diet

Ketogenic diets are the ones that promote biological ketosis through a high consumption of fat, low in carbohydrates and adequate in protein. Ketogenic diets may fight neuroinflammation by decreasing oxidative stress, increasing mitochondrial biogenesis pathways and reducing pro-inflammatory cytokine production (53). A 6-month phase II study conducted in USA that pwMS following a ketogenic diet had significant reductions in fat mass, self-reported fatigue and depression, neurological disability adipose-related inflammation, while QoL related to physical and mental health, were improved (54). Another, 3-month randomized control trial (RCT) in USA, consisting of three diet groups of pwMS (Paleolithic diet, medium-chain-triglyceride-based ketogenic diet and usual diet) showed that there were no significant clinical improvements in pwMS following a ketogenic diet, however the total sample size of the study was very small (15 pwMS) (55). A 6-month RCT conducted in Germany, showed that a ketogenic diet could reduce the expression of enzymes involved in the biosynthesis of inflammatory eicosanoids (56).

The Mediterranean Dietary Approaches to the Stop Hypertension (DASH) Intervention for Neurodegenerative Delay diet (MIND)

MIND diet is a combination of Mediterranean diet and DASH diet and it is known for its neuroprotective effects. It consist mostly of plant-based foods and limited amount of animal-derived and high in saturated fat foods, while it focuses on the consumption of berries and green leafy vegetables, which are known for their anti-inflammatory, antioxidant and neuroprotective effect (57). According Noormohammadi et al (2022) in a case-control study conducted in Iran, adherence to MIND diet was associated with lower risk of MS (58). A 3-month intervention study with MIND diet conducted in Spain in pwMS by the same researchers, showed that there was a significant decrease in fatigue and improvement in physical-related QoL, but not for the mental-related QoL. Also, in pwMS performing moderate-intense physical activity (PA), the serum levels of brain-derived neurotrophic factor (BDNF) were increased and oxidative parameters, specifically lipoperoxidation products and 8-hydroxy-2'-deoxyguanosine were decreased (59).

Paleolithic diet

The Paleolithic diet inspired by the nutritional habits during the Paleolithic era, indicates the consumption of green leafy vegetables, plant proteins, fish, eggs, unprocessed lean meat and nuts and it excludes dairy and processed food (60). A limited number of studies have evaluated the effect of the Paleolithic diet on pwMS. A 3-month RCT, consisting of three diet groups of pwMS (Paleolithic diet, medium-chain-triglyceride-based ketogenic diet and usual diet) showed that modified Paleolithic diet had significant clinical improvements in pwMS, however the sample size of the study was very small (15 pwMS) (55). A RCT of 17 pwMS in USA, showed that the Paleolithic diet could improve the fatigue symptoms and the QoL of the participants (61).

McDougall diet and Swank diet

McDougall diet focuses on the consumption of carbohydrates deriving from plants, while it is very low in fat by limiting the consumption of olive oil, meat, dairy and animal products (62). A RCT of at least 6 months conducted in USA, observed that there were significant improvements to the fatigue of pwMS, but no significant changes were noticed on brain MRI, relapse rate and disability over one year (63).

The Swank diet limits the consumption of saturated fats, while it focuses on the high consumption of fruit, vegetable and oil, since Swank observed that people consuming highly oil have a higher risk of MS (32). A 4-month RCT in USA, reported that Swank diet was associated with significant reduction of fatigue and increased QoL in pwMS (64).

Fasting Mimicking diet (FMD) and intermittent fasting diet (IF)

Fasting mimicking diet is a term used nonspecifically to describe a wide range of diets, in which a fasting state is achieved through prolonged periods of little or no calorie intake (65). Intermittent fasting diets are categorized in three types: time-restricted eating, alternate-day fasting and the 5:2 method, which consists of five designated eating days and two non-consecutive fasting days. A systematic review about the effect of IF and FMD diet on pwMS, including five studies, four RCTs and one pilot study, showed that IF could potentially have positive effects on managing symptoms and improving the QoL in pwMS (66). A 2-month RCT by Fitzgerald et al (2018) in USA, included in this review, showed that an intermittent calorie restriction diet in pwMS could reduce specific T cells subsets, too (67).

Food groups, nutrients and MS

Foods and nutrients as part of a diet contribute to the risk of MS development and disease progression in pwMS due to their nutrient content and inflammatory potential.

Fruit and vegetables (FV)

A systematic review and meta-analysis including eight observational studies regarding FV consumption and risk of MS, showed that FV consumption may have a protective effect against MS and specifically according to their dose-response meta-analysis, an increase of 100 grams of fruits per day reduced the risk of MS by 9% (68). A cohort conducted in Australia with international pwMS showed that pwMS with higher FV consumption had significantly higher health-related QoL, lower disease activity and mild disability (69). Another cohort conducted in Denmark with pwMS showed that high consumption of vegetables resulted in 32-74% reduction in symptom burden compared to low vegetable consumption (70). These outcomes may result due to the role of FV to reduce proinflammatory mediators and enhance immune cell profile (71).

Fish

Overall, there is a growing body of evidence globally to suggest a protective effect of fish consumption in risk of MS. A systematic review and meta-analysis of fish consumption and risk of MS showed that higher fish and oily fish consumption was associated with lower risk of MS (72). A case-control study included in this review (72) conducted on a Swedish population from 16 to 70 years old, showed a decreased risk of MS with the consumption of oily fish, too (73). That review included six case-control studies published prior to September 2019; since then, another analysis using two case-control studies conducted in Swedish adult populations showed consistent results with the previous studies that low fish consumption could increase the risk of MS (74). Furthermore, fish consumption and specifically oily fish has been associated with better disease-related outcomes and QoL and lower disability in people with MS (75–79). Omega-3 polyunsaturated fatty acids and fish oil contained mainly in oily fish has been shown to be neuroprotective and have a preventive role in MS (79–81). Additionally, oily fish are the main dietary source of vitamin D, which has been associated with lower MS incidence and better quality of life, symptoms severity and mental health in people with MS (82–85). A systematic review and meta-analysis including 7 clinical trials about the effect of ω -3 fatty acids and fish oil supplementation on MS has shown that they may reduce the relapsing rate, inflammatory markers and improve the QoL in pwMS (80).

Meat and animal products

The evidence about meat and risk of MS is inconclusive. A recent study has shown that non-processed red meat may have a protective effect against MS, while no statistically significant results were found for processed meat (86). Furthermore, a study by Rezaeimanesh et al (2021), showed a lower risk of MS with higher red meat and poultry consumption (87). In contrast, according to Cantoni et al (2022) people with MS have a higher total meat consumption and decreased gut

microbiota (88), while another study has reported higher disability progression in people with MS that consume meat (89). A cohort from Australia that recruited international pwMS reported that consuming no meat or dairy had higher both physical and mental QoL and lower disease activity compared to meat and dairy consumers (69). On the other hand, a cohort from Denmark in pwMS found no overall significant results between symptoms burden and meat consumption (70). Moreover, other studies have noticed higher risk of MS with higher red processed and unprocessed meat consumption (90–92) and total meat consumption including poultry (93). In general, high consumption of processed and unprocessed red meat is considered part of a pro-inflammatory diet, which has been associated with higher risk for MS (40) and increased risk of relapses in people with MS (43). As such, more evidence on the role of meat consumption and risk of MS and disease progression in MS are required.

Dairy products

Additionally, studies with different designs assessing potential associations between dairy consumption and risk of MS are controversial regarding both the effects of the consumption and the dairy subgroups. Some studies have reported that total dairy intake above recommended levels is associated with increased risk of MS or higher risk of relapses and/or clinical symptoms worsening in people with MS (94,95), other studies found no effect (96,97), while others found a protective effect (98,99). Concerning dairy subgroups, some studies have shown a decreased risk of MS and yogurt consumption (96,99). This may be explained due to the probiotics concentration in yogurt, which have been associated with lower risk of MS, slower disability progression, better mental and general health and decreased inflammation in people with MS (100–102). Conversely, according to the Nurses' Health Study and the Nurses' Health Study II, milk was associated with increased risk of MS in women that consumed whole milk three or more times per day (103), while an inverse association was found in a study from Iran (99).

Alcohol

An analysis using data from the UK Biobank found no association between alcohol consumption and risk of MS (104), while a case-control analysis from Sweden showed a 20% reduction of risk of MS among alcohol consumers compared to never-consumers. Authors supposed that the protective effect of alcohol against MS may be more predominant in individuals with the risk-increasing HLA allele, DRB1*15:01 (105). A case control from Denmark had similar results. Particularly, it was noticed that alcohol consumption in adolescence was associated with decreased risk of MS (106). According to a review by Fahim et al (2020), it is concluded that most of the MS therapies target to reduce inflammation and suppress the immune system, acute and moderate alcohol consumption

suppresses the immune system and its response to pathogens, while chronic alcohol consumption suppresses the immune system, too, however it increases the immune system responses to pathogens (107). A case-control study in Sweden showed that low and moderate alcohol drinking had better outcomes regarding the RRMS and disability mostly among women, compared to nondrinking, while there were no significant outcomes with high alcohol intake (108). However, it should be considered that alcohol consumption is an exposure to multiple health-related risks and diseases and there is no therapeutic dosage for the immune system suppression (107).

Coffee

Overall, studies on the relationship between coffee consumption and risk or progression of MS show protective results (35,109). For example, two case-control studies in Sweden and USA showed that a high coffee consumption of over 900 ml per day was associated with reduced risk of MS (110). These positive associations may be explained due to the neuroprotective properties of the caffeine that suppressed the production of proinflammatory cytokines (110). However, Massa et al (2013) using data from the Nurses' Health study from USA in women did not find any significant association between coffee or caffeine intake risk of MS (111). A cross-sectional analysis with pwMS from Belgium and the Netherlands showed that participants drinking coffee daily had an extension of 4 years to arrive at Expanded Disability Status Scale (EDSS) 6 level compared to pwMS that never drank coffee (112).

Olive oil

In animal studies using rats injected with an emulsion to reproduce the experimental model of MS, reported that extra virgin olive (EVOO) could reduce motor symptoms, oxidative damage and weight loss (113,114). Olive oil, especially EVOO, is recognized for its anti-inflammatory properties because of its content in phenolic compounds (115,116).

Vitamin D

Even though, vitamin D can be produced mostly through sunlight exposure and it is not commonly found in food sources, but in oily fish, it is one of the main risk factors associated to risk of MS and disease activity, when in deficiency. Consequently, studies support that the MS is more prevalent at higher latitudes and in areas with low exposure to ultraviolet light. New Mendelian randomization analyses have suggested a causal relationship between low vitamin D levels and risk of MS. Due to the autoimmune nature of MS, the role of vitamin D for the prevention of MS could be attributed to its important role in lymphocyte activation and proliferation, T-helper cell differentiation, tissue-specific lymphocyte homing, the production of antibody isotypes and the regulation of the immune response (117). An analysis from the Nurses' Health Study in women in USA, showed that women

with high dietary intake of vitamin D around 700 IU/day through food had by 33% lower incidence of MS and women with vitamin D supplement use (≥ 400 IU/day) had a 41% lower risk of MS (118).

Sodium

Salt intake has controversial results about its role in MS. There are studies suggesting that high salt intake above 5 grams/day could have a negative result in the onset of the disease through the induction of pathogenic phenotype of Th17 cells, especially nowadays with the increasing adherence to a Western diet, high in processed meals and fast food (119). According to a 2-year cohort in Argentina with pwMS, it was found that high sodium intake was correlated with increased exacerbation rate and disease duration (120).

Other nutrients

Multiple other nutrients have been studied related to their potential effect on MS, however without robust evidence and adequate data. For instance, the role of vitamin B complex and in particular vitamin B12 is discussed due to their anti-inflammatory role in methylation process, involvement in myelination and remyelination, hence the potential beneficial results on MS symptoms (121). Furthermore, a systematic review about the link between riboflavin and MS concluded that pwMS in RCTs and case-control studies using riboflavin supplementation or having high dietary riboflavin intake had improvements in neurological motor disability. These results may be because of the oxidative damage that riboflavin deficiency causes (122). Instead, a systematic review conducted about the efficacy of melatonin supplementation in MS, showed that melatonin could increase the oxidative stress and inflammation, while few studies showed positive outcomes about sleep conditions, cognitive outcomes and fatigue in pwMS (123). A 4-month RCT in Iran testing the effect of probiotic supplementation with *Saccharomyces boulardii* on the clinical outcomes of MS showed that the group receiving the supplement had significantly decreased inflammatory marker high-sensitivity C-reactive protein and the serum antioxidant capacity increased significantly compared to the placebo. Additionally, the group receiving the supplement had improvements in their QoL, decreased pain intensity and fatigue severity and somatic and social dysfunctions (102). Furthermore, a 3-month RCT showed that the supplementation of 500 mg/day of coenzyme Q10 could reduce oxidative stress and increase antioxidant enzyme activity in pwMS (124).

Lifestyle factors, inflammation and MS

Physical activity (PA)

A systematic review and meta-analysis by Du et al (2024) included forty RCTs to assess the effect of exercise in pwMS. Different types of PA were studied in the eligible publications such as yoga, pilates, treadmill, walking, strength training, water sports and others. The authors found that

overall, PA in pwMS could significantly improve balance, walking ability, fatigue and QoL. According to subgroup analysis, aerobic exercise, resistance exercise and multicomponent training were all effective to fatigue, but the greatest results were found with resistance exercise (125). A more recent systematic review from 2022 including 43 studies with different designs (clinical trials, cross-sectional and cohort studies) concluded that exercise could affect positively the disability according to the Multiple Sclerosis Functional Composite (MSFC), but EDSS score, lesion load and brain volume may remain unchanged (126). Unfortunately, PA among pwMS remains relatively low compared to healthy individuals with men having higher PA than women (127–129). Low PA is associated with persistent systemic low-grade inflammation (130), instead a systematic review and meta-analysis by Bellisario et al (2024) reported that specifically regular aerobic exercise could reduce and positively modulate inflammation in pwMS (131). Regarding risk of MS, in a Mendelian randomization analysis using data from the UK Biobank, higher moderate PA was associated to lower risk of MS (132).

Sleep quality

Sleep disturbances and disorders are common in pwMS and they are significantly higher than in the general population or other chronic diseases. Additionally, sleep problems are more prevalent in women with MS (133). The most frequent sleep disorders in pwMS are insomnia, sleep apnea, restless legs syndrome, narcolepsy and rapid eye movement (134). A systematic review and meta-analysis including 46 studies, concluded that sleep quality and insomnia are strongly associated with fatigue in pwMS, but not sleep duration and number of awakenings (135). Additionally, poor sleep in pwMS is strongly associated with worse health-related quality of life in the Australian MS Longitudinal Study (136). A cross-sectional study from Slovakia in pwMS found that restless legs syndrome was the main factor for poor sleep, fatigue and daytime somnolence in the participants recruited (137). Moreover, depression and anxiety play an important role in sleep disturbances in pwMS (138). The bad sleep quality in pwMS may play a role in their inflammation status, since according to a systematic review and meta-analysis, which included 72 studies, concluded that sleep disturbance are associated with increases in markers of systemic inflammation (139). Finally, low sleep quality and insufficient sleep during adolescence is associated to a higher risk of MS according to a case-control study conducted in Sweden (140).

Smoking

Smoking is one of the main known environmental, lifestyle factors associated with a higher risk of MS (141). Due to its pro-inflammatory effect and inflammation caused to individuals by smoking,

the risk of MS is increased (141). Cigarette smoke due to its reactive oxidative substances caused by burning, enhances inflammatory and suppressive effects on immune cells by triggering oxidative stress, damaging airway cells, skewing immune responses by suppressing Th1 and promoting Th2/Th17 pathways and finally, by weakening the immune system defenses against infections like pneumonia and tuberculosis (142). According to a review, including studies from multiple countries, such as Sweden, Norway, Israel, the UK and USA reported that smokers have 1.5 higher risk of MS than non-smokers. Furthermore, some studies included in this review, suggested a dose-response effect of smoking on risk of MS (20). Concerning disease progression and smoking, most of the studies, but not all have found a relationship. For instance, multiple studies have found that it is more likely smokers with MS to convert from RRMS to SPMS (143–145). Additionally, smokers have worse disability scores and in the cross-sectional study by Healy et al (2009) with longitudinal follow-up in USA, brain MRI T2-weighted lesion volume of pwMS increased faster and brain parenchymal fraction decreased faster in smokers than nonsmokers (145).

Mental health

Generally, studies have shown that the QoL of pwMS is lower than the general population (146). A systematic review and meta-analysis on stress and MS, including 30 studies, concluded that stress may have a minor to moderate effect on disease onset, inflammatory activity and progression of the disease (147). Depression and anxiety are the most common neuropsychiatric symptoms in pwMS, with a prevalence of 25-65% and 20-54%, respectively. Usually, they exist in the person before the diagnosis of MS and they lead to faster disability and low QoL. Both depression and anxiety are linked to oxidative stress, mitochondrial dysfunction, neuroinflammation promoting the development of functional and brain lesions. Depression in MS has been associated with cognitive dysfunctions, too (148). Moreover, cigarette smoking, alcohol dependence and depression and anxiety have been linked in pwMS, while there are studies that support a causal smoking-depression relationship (149–151).

Chapter 2 Summary of the methodology of the EXPOSITION study protocol

Study objectives

The EXPOSITION study is a cross-sectional investigation designed to explore how the external exposome, including environmental exposures, urbanization, air pollution, occupational factors, and lifestyle habits, affects biomarkers of oxidative stress and inflammation in people with multiple sclerosis (pwMS).

The primary aim is to examine how microRNA (miRNA) expression, a key marker of internal biological responses, varies in relation to these external factors. The analysis will account for potential confounders, including age, sex, anthropometrical measurements, vitamin D levels, medical history, nutrition and lifestyle habits and other biomarkers such as serum cytokines and neurofilament levels.

The secondary aim is to investigate changes in the gut and nasal microbiota as additional indicators of oxidative stress and inflammation. Microbiota will be evaluated for diversity (alpha-diversity), differences in community composition (beta-diversity), and relative abundance of specific bacterial taxa across different exposome conditions.

Overall, the study seeks to link environmental and lifestyle exposures to molecular and microbial changes that may contribute to oxidative stress and inflammation in MS, providing insight into how external factors influence disease biology.

Population, Inclusion Criteria, and Informed Consent

To achieve the objectives of the EXPOSITION study, 200 people pwMS attending the Multiple Sclerosis Center of the IRCCS Fondazione C. Mondino in Pavia, Italy, were set as sample size goal.

The inclusion criteria were:

- Age ≥ 18 years
- Diagnosis of MS according to the 2017 McDonald criteria (13)
- Relapsing-remitting (RR) MS (152)
- Routine clinical examinations scheduled during the study periods (March–April 2024 and October–April 2025)
- Residence in the provinces of Pavia or Milan
- Signed informed consent

Individuals who refused to participate were excluded. Potential participants were screened by a neurologist specialized in MS to ensure all criteria are met. Participants were initially selected from the Pavia Database of Multiple Sclerosis, which includes 1,400 patients, has been active since 1990,

and is part of the Italian Multiple Sclerosis Register since 2015. The study adheres to the Declaration of Helsinki, with written informed consent obtained from all participants. Ethical approval was granted by the Ethical Committee of IRCCS Policlinico San Matteo (protocol number: 0040023/23; approved 25/07/2023). The study protocol is registered on ClinicalTrials.gov (NCT06325358).

Exposure and outcomes variables collection

All participants, as outlined in Figure 1, provided information on their social, demographic, and clinical characteristics, as well as general and specific external exposome factors (Figure 2). Internal exposome factors, including primary and secondary outcomes, were assessed through blood, stool, and nasal swab samples. Each participant was also linked to their municipality of residence, allowing collection of ecological data on air pollution and urbanization associated with their geographic area.

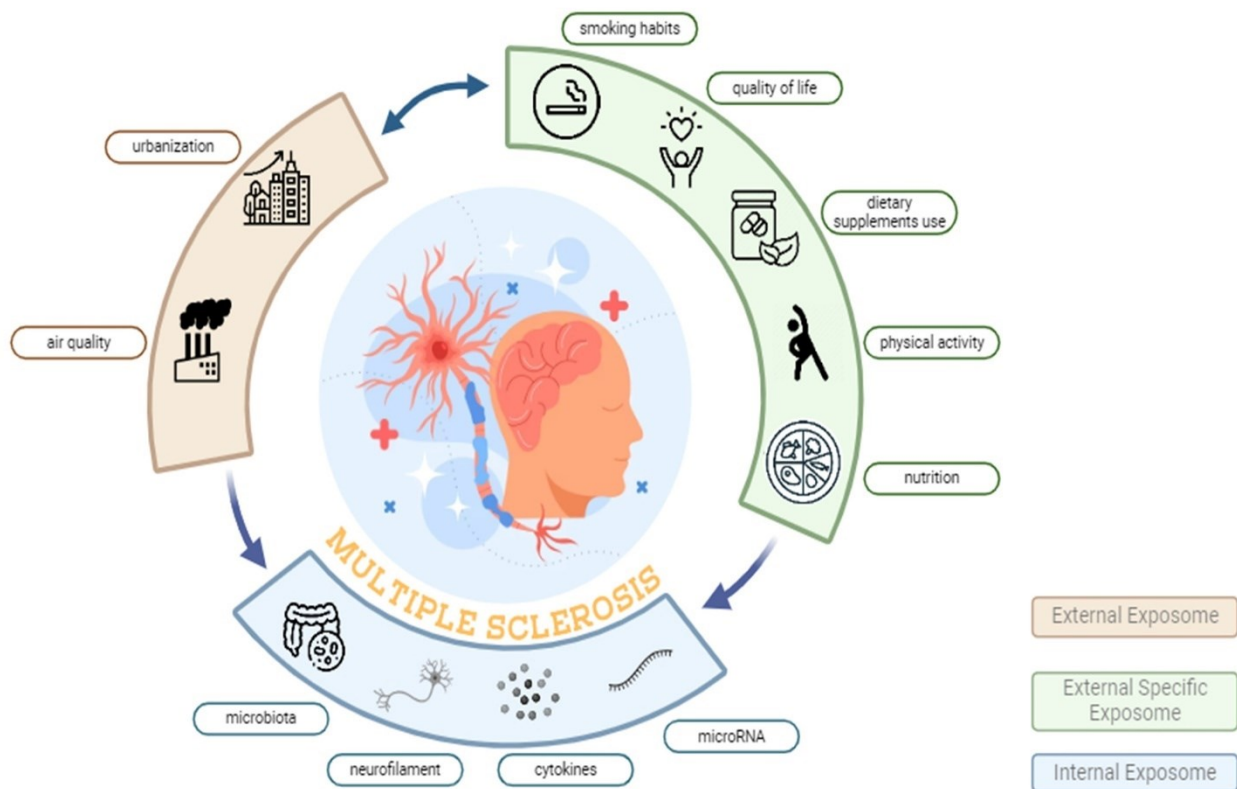


Figure 1 External and internal exposome factors assessed in EXPOSITION study

Social, Demographic, and Clinical Data

Social and demographic data included age, sex, ethnicity, date and place of birth, civil status, education level, employment status and current residence were reported. Clinical data included information on disease onset and diagnosis (date of onset, date of diagnosis, presence of oligoclonal bands), disease duration, MS phenotype, date of the last relapse, and details from the most recent MRI (new/enlarging T2-weighted lesions, Gadolinium-enhancing lesions), as well as No Evidence of

Disease Activity (NEDA3) status were assessed by neurologists. Information on disease-modifying treatments included the number and type of previous therapies, reasons for discontinuation, and start date of the ongoing treatment, along with symptomatic treatments for MS or other conditions. Clinical disability was quantified using the EDSS (153). All data were anonymized and collected in REDCap.

General and specific external exposome factors

Air Quality Evaluation

Two- and three-dimensional fields of major atmospheric components were obtained from state-of-the-art models and satellite observations. The CAMS and CHIMBO air quality models provided spatial data (8–10 km resolution) on particulate matter (PM₁₀, PM_{2.5}), black carbon (BC), nitrogen dioxide (NO₂), and ozone (O₃). Hourly, daily, weekly, and seasonal concentrations since March 2022 were collected to characterize air quality in the Milan and Pavia provinces. Additionally, Sentinel-5P satellite products from the TROPOMI sensor were used to obtain tropospheric columns of O₃, SO₂, NO₂, and CO at 3 km × 3 km resolution (resampled to 1 km × 1 km when needed). Missing data were filled and noise reduced using post-processing techniques. Air pollution data were linked to participants' municipalities via geolocation from residence addresses. A source apportionment analysis using chemical transport modeling was performed to estimate contributions from urban traffic and other emission sources. Questionnaire data on daily time spent indoors/outdoors and near/far from home were also incorporated to refine individual exposure estimates.

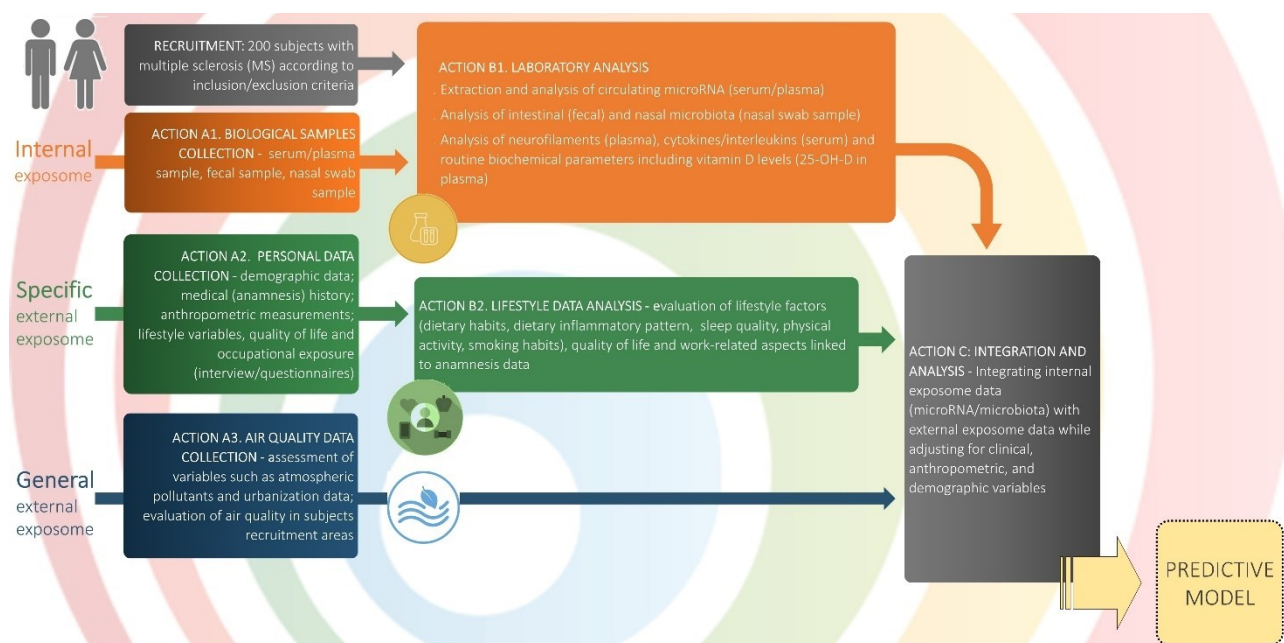


Figure 2 Flowchart and actions of EXPOSITION study

Urbanization

The European Degree of Urbanisation (DEGURBA) classification, based on the 2020 census, was adopted to stratify municipalities in the provinces of Pavia and Milan as cities, town-suburbs, or rural areas (154). According to this system, Pavia and Milan (with 47 other municipalities) were classified as cities, 27 municipalities in Pavia and 77 in Milan as town-suburbs, and 158 in Pavia and 8 in Milan as rural areas. DEGURBA follows country-specific definitions; in Italy, a city is defined by ISTAT as a local administrative unit with $\geq 50,000$ inhabitants and a high-density population cluster.

Nutritional status and lifestyle assessment

The assessment of the nutritional status of study participants included both instrumental measurements to evaluate body weight and composition, as well as analyses of dietary intake. These involved examining eating habits and food consumption frequencies using specifically validated questionnaires.

Particularly trained dietitians/nutritionists conducted anthropometric and body composition measurements under standardised conditions. Body weight (kg) were measured with participants in their underwear, using a calibrated balance scale with an accuracy of ± 100 g. Height (cm) was recorded, and the Body Mass Index (BMI) was calculated as weight (kg) divided by height (m) squared (155).

Waist circumference (WC) was measured to the nearest centimetre using a flexible steel tape with participants standing upright, arms crossed on opposite shoulders, and after a gentle exhalation. The measurement will be taken at the midpoint between the lowest rib and the upper lateral margin of the right iliac crest. The waist-to-height ratio (WHtR), an established marker of abdominal adiposity with a cut-off of <0.5 (156), were determined.

Body composition, including phase angle, was assessed using bioelectrical impedance analysis (BIA) with the BIA 101 BIVA[®] PRO device (157). Phase angle, a reliable indicator of cellular health and nutritional status (158), were analysed alongside other parameters to provide a comprehensive evaluation of participants' body composition (159).

Regarding lifestyle habits, the trained personnel will also collect lifestyle variables, using previously validated questionnaires (160–164): dietary habits and frequency of consumption; physical activity level; smoking habit; quality of sleep; level of stress and Quality of Life (QoL). The dietary habits of the participants will be evaluated with the use of a validated food frequency questionnaire (FFQ) adapted to the Italian population (162) and adjusted based on current food trends, such as the consumption of plant-based milk and meat. Detailed information about the daily food consumption

of pwMS will be gained through a 24-hour dietary recall. Dietary supplement use was assessed based on the type and nutrients included in the dietary supplements, their dose and their frequency of use.

The Dietary Inflammatory Index (DII) was used to quantify the overall effect of diet on inflammatory potential. The DII was calculated based on the daily food consumption data of the participants to calculate the food parameters included in the index. Specifically, the food parameters included were alcohol, caffeine, onion, green/black tea, energy, carbohydrates, protein, fats (total fat, cholesterol, monounsaturated fatty acids, polyunsaturated fatty acids, n-3 fatty acids, n-6 fatty acids, saturated fat, trans fat), fibre, vitamins (vitamin B12, vitamin B6, b-carotene, folic acid, niacin, riboflavin, thiamin, vitamin A, vitamin C, vitamin D, vitamin E), minerals (iron, magnesium, selenium, zinc) and flavonoids (flavanols/flavan-3-ol, flavones, flavonols, flavonones, anthocyanidins, isoflavones) (165). Food parameters such as garlic, ginger, saffron, turmeric, pepper, thyme/oregano and rosemary were not considered for the calculation of DII, due to the difficulty of measuring their dietary intake from the 24h recalls. The scoring algorithm ranges from -1, which represents a maximally anti-inflammatory factor, to +1, which represents a maximally pro-inflammatory factor. Finally, all food parameters were summed to the overall DII score for the diet of everyone (165).

The MEDI-LITE score was used for the assessment of adherence to the Mediterranean dietary model (163). This scoring system is based on a questionnaire, which investigates the frequency of consumption of nine classes of food; the final score varies from 0 (low adherence) to 18 (high adherence); overall, subjects who obtain a score higher than 9 will have a significantly increased adherence to the Mediterranean Diet (163).

Physical activity levels was evaluated by using the short form of the International Physical Activity Questionnaire (IPAQ) (161), which includes 7 items concerning the last 7 days' levels of moderate, vigorous and sedentary physical activity. The metabolic equivalent of tasks (MET) was calculated based on the frequency and duration of physical activity and expressed as MET-minutes per week (MET-min/week) (161). The calculation followed the formula: METs = MET level × minutes of activity × events per week (161).

Based on the total MET score, participants were classified into three categories of physical activity: low, moderate, and high (161). Individuals were considered to have a low level of physical activity if they don't meet the criteria for the moderate or high categories (161). The moderate activity level included participants who reported vigorous-intensity activity on at least three days for a minimum of 20 minutes per day; or moderate-intensity activity and/or walking on at least five days for a

minimum of 30 minutes per day; or any combination of walking, moderate- or vigorous-intensity activities on five or more days, reaching a total of at least 600 MET-minutes per week (161). The high activity level comprised individuals who reported vigorous intensity activity on at least three days and accumulated a minimum of 1500 MET-minutes per week, or any combination of walking, moderate-, or vigorous-intensity activities performed on seven or more days, achieving a total of at least 3000 MET-minutes per week (161).

Sleep quality was measured by the Pittsburgh Sleep Quality Index (PSQI) questionnaire (160), consisting of 9 multiple-choice items divided into 5 different components related to subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication and daytime dysfunction. The global PSQI score was obtained by considering the scores of all the components, and it ranges from 0 to 13. The PSQI cut-off of 5 or above indicates poor sleep quality (160).

Cigarette smoking habits was documented by classifying participants as current smokers, former smokers, or never smokers. For current smokers, the number of cigarettes smoked per day and the duration of smoking in years was recorded. Former smokers were asked to report the number of years they smoked and their average daily cigarette consumption during that period.

Quality of life was evaluated by Multiple Sclerosis Quality of Life-29 (MSQOL-29) questionnaire (164), a multidimensional health-related quality of life measure that combines both generic and MS-specific items into a single instrument. The questionnaire consists of 29 items, divided into 7 subscales related to physical functioning, pain, emotional well-being, energy, cognitive function, health stress, sexual function, change in health, social function, health perception and overall quality of life (164).

Internal exposome variables

Blood samples were collected by trained research nurses without requiring fasting and anonymized using a progressive code with participants' sex and date of birth. Samples were sent to the Mondino Molecular Genetic and Neurogenetic Laboratory for pre-analytical processing, including centrifugation, separation, and storage at -80°C. Serum and plasma were processed within two hours of collection, aliquoted, and stored for subsequent analysis. Blood analyses included miRNA expression, neurofilament proteins (NfL), pro- and anti-inflammatory cytokines, and 25(OH)D levels.

Stool samples were collected by participants following detailed instructions, ensuring absence of fever, gastroenteritis, diarrhea, or recent antibiotic use. Nasal swabs were collected using flexible

flocked swabs in transport medium. Both stool and nasal samples were anonymized and stored at -80°C until microbiota analysis.

MicroRNAs expression levels (primary outcome)

The differential expression of seven selected circulating plasma miRNAs, previously identified as deregulated in MS, was measured in each participant. Serum samples were collected, frozen at -20°C, and later thawed for RNA extraction. miRNAs were reverse-transcribed and analyzed using Real-Time qPCR with TaqMan or SYBR Green assays. Relative expression levels were calculated using the $\Delta\Delta CT$ method according to Livak et al., comparing CT values to endogenous controls and control samples to determine fold changes. Statistical analyses were performed to assess differences in miRNA expression across different external exposome variables (166).

Gut and nasal Microbiota analysis (secondary outcomes)

Stool and nasal samples were stored at -80°C until genomic DNA extraction. DNA was isolated using the Maxwell® RSC Faecal Microbiome and Pathogen Total Nucleic Acid Kits with an automated extractor. DNA quality and quantity were assessed using TapeStation 4200 and Qubit 4 fluorometer. Sequencing libraries were prepared with an NGS metabarcoding approach, amplifying the V3-V4 regions of the 16S rRNA gene, indexed with dual Nextera® XT indexes, and sequenced on an Illumina NextSeq1000 platform. Raw reads were processed using a pipeline that merged paired-end reads, filtered low-quality sequences, and generated Amplicon Sequence Variants (ASVs) via DADA2 (167). Taxonomic classification was performed using the RDP classifier (168) against the SILVA database (169). Alpha-diversity metrics (Shannon, Chao1, observed species, Faith's phylogenetic diversity) and beta-diversity (unweighted and weighted UniFrac) were calculated (170). Microbial composition was expressed as relative abundances from phylum to genus, with nasal microbiota further characterized to the species level for dominant genera (e.g., *Corynebacterium*, *Moraxella*, *Haemophilus*).

Neurofilament proteins and pro-and anti-inflammatory cytokine levels assessment

Plasma neurofilament light chain (NFL) levels were quantified using the Simple Plex assay on the Ella microfluidic immunoassay platform, following the manufacturer's protocol. Samples were diluted 1:2, and the platform automatically performed triplicate measurements. The assay had a lower limit of quantification of 2.70 pg/mL and an upper limit of 10,290 pg/mL (171). Serum cytokines and growth factors, including IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IL-22, IL-23, TGF- β , GM-CSF, TNF- α ,

IFN- γ , and CXCL10, were measured using Simple Plex assays and commercial ELISA kits, with concentrations reported in pg/mL (172).

Vitamin D levels assessment

Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured using the Elecsys® Vitamin D total III assay (Roche Diagnostics) on a Cobas Pure integrated analyzer. Fasting venous blood samples were collected, processed, and stored at -80°C until analysis. The assay, an electrochemiluminescence competitive binding immunoassay, quantified both 25(OH)D₂ and 25(OH)D₃, with validated sensitivity, linearity, and precision parameters. Vitamin D status was classified according to SIOMMS cut-offs: severe deficiency (<10 ng/mL), deficiency (10–20 ng/mL), insufficiency (20–30 ng/mL), adequacy (30–100 ng/mL), and toxicity (>100 ng/mL) (173,174).

Statistical analysis

Sample size calculation

To define the sample size, a bioinformatic analysis of public GEO databases (e.g., GSE61741, GSE31568, GSE17846) was conducted to identify diagnostic miRNAs deregulated in MS. Ten candidate miRNAs were identified, with one consistently altered across all datasets. Predicted target genes (n=26) were identified through miRTarBase and stratified by Reactome pathway analysis into inflammation, oxidative stress, lipid metabolism, cellular processes, and myogenesis. For the study, seven selected miRNAs were chosen as outcomes, with their differential expression (ΔCT) modeled in relation to external exposome factors (air pollution, urbanization, lifestyle, and quality of life). Negative binomial GLMs were planned to test mean ΔCT differences across exposure groups, including covariates (175). The minimum required sample size to detect a fold change of 1.1–4.5 with 80% power was 168 pwMS. To account for a ~15% dropout at blood sampling, 200 pwMS were initial goal of sample to be recruited.

Data integration and description

Health, clinical, and exposome data were stored in a unified EXPOSITION database, linked through a pseudonymized code for each participant. Data included absolute and relative miRNA expression, alpha- and beta-diversity indices, relative microbial abundances, and air pollution and urbanization measures assigned by municipality of residence. Quality control and missing data checks were performed. Analyses were conducted using Stata 17, R (including ExposomeShiny), and QIIME according to predefined research protocols (166,176). The first set of analyses described

participants, exposome characteristics, and biological data. Socio-demographic, internal, and external exposome variables were summarized using means, standard deviations, medians, interquartile ranges, or frequencies, depending on variable type. Log transformations were applied to non-normally distributed exposure variables. Data distributions were visualized using boxplots and violin plots, grouped by categorical exposures when appropriate.

Exposome clustering, correlation and association analyses

Exposome patterns were explored using hierarchical clustering and principal component analysis. Correlations were assessed with Pearson, Spearman, or Cramer coefficients. Associations between external exposures and internal outcomes (miRNA, microbiota) were tested using regression models (negative binomial, mixed, or linear), with FDR correction for multiple testing. Microbiota diversity was evaluated through non-parametric tests and PERMANOVA, while the most informative exposures were further modeled with lasso or elastic net penalized GLMs.

Methods of current PhD analysis

For the specific analysis, the nutritional and lifestyle methodology and the methodology related to 25(OH)D analysis have been used as mentioned in the protocol. The cut-offs used for the categorization of variables are described in table 1. Due to the non-normal distribution of the continuous variables, they are expressed both as medians and interquartiles (IQRs), while the categorical variables are expressed as number of pwMS and percentages. As regards the statistical analysis, when variables were categorical Fisher's exact test was used and when continuous Wilcoxon rank sum test was used for sex comparisons, while when the comparisons were made in more than two groups and the variables were categorical Fisher's exact test for small samples and Pearson's Chi-squared test for larger samples were used and when continuous Wilcoxon rank sum test was used. For the comparison of phase angle and DII in groups, the variables were divided equally in tertiles. Correlations among variables were tested with the non-parametric Spearman's rho test. For testing the associations between phase angle (continuous variable), DII (continuous variable) and the rest of variables, Least Absolute Shrinkage and Selection Operator (LASSO) regression was used, which is a type of linear regression used for variable selection and regularization with the score to improve prediction accuracy and interpretability (177). Missing values were present in 11.6% of cases (19 participants without nutritional data) and they were excluded from the analysis. The remaining missing values were handled with pairwise deletion

when possible or listwise deletion, when necessary, in regression. P-value was set to 0.05 and the statistical analysis was conducted with R studio.

Table 1 Cut-off definitions of main variables used in the nutritional and lifestyle evaluation

Variable	Cut-off	Explanation
BMI (178)	<18.5	Underweight
	18.5-24.9	Normal weight
	25-29.9	Overweight
	>30	Obese
WhtR (179)	≥0.5	Increased risk of central obesity and related health problems
EDSS (180)	>4	Advances disability
MSQoL-29 physical (164)		Higher score, higher physical-related quality of life
MSQoL-29 mental (164)		Higher score, higher mental-related quality of life
DII (165)	<0	Anti-inflammatory diet
	>0	Pro-inflammatory diet
IPAQ (181)	< 700	Low PA
	700-2519	Moderate PA
	>2519	High PA
PSQI (160)	<5	Good sleep quality
	≥5	Poor sleep quality
Medi-Lite (163)	<9	Poor adherence to Mediterranean diet
	≥9	High adherence to Mediterranean diet
25(OH)D (173,174)	<12 ng/ml	Deficiency
	12-30 ng/ml	Insufficiency
	≥30 ng/ml	Sufficiency

BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; WhtR: Waist-to-Height Ratio; MSQoL-29: Multiple Sclerosis Quality of life 29 questionnaire; DII: Dietary Inflammatory Index; IPAQ: International Physical Activity Questionnaire; PA: Physical Activity; PSQI: Pittsburgh Sleep Quality Index; 25(OH)D: 25-hydroxy vitamin D

Chapter 3 Objectives

This PhD thesis is part of the EXPOSITION study; a cross-sectional study aiming to study the whole exposome (external, specific external and internal) in Italian pwMS with RRMS as the most common type of MS (4) living in the provinces of Pavia and Milan. As exposome is defined any exposure of an individual during the lifetime and how they relate to health and disease (182). As mentioned above, the role of the external exposome and especially the available data about the role of nutritional status and diet in MS are scarce. Especially studies and analyses about nutrient intake, common phase angle values found in this population and DII are mostly unavailable particularly in pwMS. The main objectives of this thesis according to literature gaps are the followings:

1. Extended assessment of nutrition and lifestyle habits of pwMS and analysis of nutrient intake for the total population and by making sex-specific comparisons. A recent review by Barbuti et al (2025), reported that there is some evidence for few sex-specific differences about the risk of MS and the progression regarding the known risk factors such as obesity, smoking and vitamin D status, however no study till now has reported the sex-specific differences regarding the total external exposome of pwMS that could help to create individualized, effective and safer therapeutic strategies (8).
2. Extended assessment and comparisons of nutrition and lifestyle habits of pwMS and nutrient intake stratified by phase angle tertiles and evaluation of potential associations between phase angle and the other nutrition and lifestyle variables analyzed. Inflammation influences the phase angle values, with lower phase angle indicating chronic inflammation and some studies indicate that phase angle could be used as an indicator of oxidative stress (183). Even though, MS is an inflammatory disease that reduces the moving ability of pwMS and possibly they PA levels, no phase angle range has been defined for this population and the potential variables that influence these values.
3. Extended assessment and comparisons of nutrition and lifestyle habits of pwMS and nutrient intake stratified by DII tertiles and evaluation of potential associations between DII and the other nutrition and lifestyle variables analyzed

Chapter 4 Results

Overall results about nutrition and lifestyle habits and sex-specific differences in pwMS

In total 150 pwMS were enrolled and given blood samples, while most of the nutritional and lifestyle assessment data were available for 131 pwMS. The mean (SD) age of the population was 46 (13) years and the majority of them was female (67%). The highest percentage of pwMS had finished high school education, while the education of women was significantly higher than men ($P=0.048$). The EDSS of the population was relatively low with a median of 1.5 for both males and females. The median (IQR) BMI was 24.2 (21.1–26.7), so close to overweight cut-off and did not differ between males and females. Most of the participants had a WhtR ≥ 0.5 indicating increased risk of central obesity and related health problems. The median (IQR) PhA was 5.35 (4.9–5.8)^o, with statistically significantly higher values for males (P -value=0.006). Most of pwMS were non-smokers at the moment of the enrollment (52.7%). The median (IQR) MSQoL-29 physical was 49 (11–81), with high variation among the participants, but no significant differences regarding sexes, while the median (IQR) MSQoL-29 mental was 61 (19–97) and it was significantly worse in women (P -value <0.001). The median (IQR) DII was 4.12 (2.41–5.14) indicating a highly pro-inflammatory diet without differences between the sexes, while only 7 (5.3%) of the participants had an anti-inflammatory diet. The median (IQR) Medi-Lite score was 11 (9–12) and it was above 9 for both men and women showing a good adherence to the Mediterranean diet. Even though, PA was not statistically different between men and women ($P=0.053$), it is shown that 26% of men had high PA in contrast to women that was 10% according to IPAQ. The median (IQR) of sleep quality according to Pittsburg score was 5 (4–8) reporting a bad sleep quality, but significantly worse among women ($P=0.010$). Finally, interestingly median (IQR) 25(OH)D were 22 (15–35), showing insufficiency of plasma vitamin D levels and they were statistically significantly different between sexes with women having higher values than men ($P=0.005$), even though 51.2% of the participants used vitamin D supplements and 73.1% of them were females (Table 1).

Table 2 Basic characteristics, anthropometrical measurements and nutrition and lifestyle indexes for the total, male and female population expressed as median (IQR)

	Total (N=150)	Male (n=50)		Female (n=100)		P-value*
Age (years)	46 (35–57)	47 (13.8)	47.1 (34.2–59.3)	44.8 (12.2)	45.5 (35.5–54.9)	0.3
Sex (n%)						
Female	100 (67)	0 (0)		100(100)		
Male	50 (33)	50(100)		0(0)		
Educational level (n%)						0.048
Middle school	25 (19)	10 (23.3)		15 (17.1)		
High school	55 (42)	23 (53.5)		32 (36.4)		

Bachelor's degree	45 (34)	10 (23.2)	35 (39.8)	
Master's degree	6 (5)	0 (0)	6 (7.1)	
EDSS	1.5 (1-2)	1.5 (1-2)	1.5 (1-2.5)	0.2
≤4 (n%)	134 (89)	46 (92)	88 (88)	0.5
>4 (n%)	16 (11)	4 (8)	12 (12)	
New lesions (n%)				0.2
No	140 (93.3)	49 (98)	91 (91)	
Yes	10 (6.7)	1 (2)	9 (9)	
Growing lesions (n%)				>0.9
No	145 (97.3)	49 (98)	96 (97)	
Yes	4 (2.7)	1 (2)	3 (3)	
Active lesions (n%)				>0.9
No	146 (98)	49 (98)	97 (98)	
Yes	3 (2)	1 (2)	2 (2)	
NEDA-3 (n%)				0.1
No	11 (7)	1 (2)	10 (10)	
Yes	139 (93)	49 (98)	90 (90)	
Weight (kg)	65 (57–81)	80 (65.5–86)	61.5 (3.1–69.6)	<0.001
Height (m)	1.64 (1.59–1.72)	1.75 (1.7–1.8)	1.6 (1.6–1.7)	<0.001
BMI (kg/m ²)	24.2 (21.1–26.7)	24.4 (21.5–27.1)	23.4 (20.8–26.5)	0.4
Underweight (n%)	9 (6.9)	2 (4.7)	7 (8)	0.7
Normal weight (n%)	69 (53)	21 (49)	48 (55)	
Overweight (n%)	31 (24)	13 (30)	18 (20)	
Obese (n%)	22 (17)	7 (16)	15 (17)	
WhtR	0.51 (0.45–0.57)	0.51 (0.46–0.58)	0.5 (0.44–0.55)	0.2
<0.5 (n%)	58 (47)	18 (43)	40 (49)	0.5
≥0.5 (n%)	65 (53)	24 (57)	41 (51)	
WC	83 (75–94)	90.5 (81–100)	79.5 (70–89)	<0.001
PhA ^o	5.35 (4.9–5.8)	5.6 (5.1–6.3)	5.3 (4.8–5.6)	0.006
Smoking status (n%)				
Non-smoker	69 (52.7)	18 (41.9)	51 (58)	0.075
Smoker	34 (26)	11 (25.6)	23 (26.1)	
Ex-smoker	28 (21.3)	14 (32.5)	14 (15.9)	
MSQoL-29 physical	49 (11-81)	47.3 (39–58.9)	48.8 (44.3–58.9)	0.2
MSQoL-29 mental	61 (19-97)	70.5 (60.8–77.1)	55.7 (47.9–68.5)	<0.001
DII	4.12 (2.41–5.14)	3.67 (2.25–5.14)	4.16 (2.6–5.14)	0.2
Anti-inflammatory (n%)	7 (5.3)	4 (9.3)	3 (3.4)	0.2
Pro- inflammatory (n%)	124 (95)	39 (91)	85 (97)	
Medi-Lite	11 (9-12)	10 (8-12)	11 (9-12)	0.3
Low adherence (n%)	25 (19)	11 (25.6)	14 (15.9)	0.2
High adherence (n%)	106 (81)	32 (74.4)	74 (84.1)	
IPAQ (METs)	693 (231–1,845)	1,032 (219–2,619)	653 (231–1,274)	0.2
Low PA (n%)	69 (53)	18 (42)	51 (58)	0.053

Moderate PA (n%)	42 (32)	14 (33)	28 (32)	
High PA (n%)	20 (15)	11 (26)	9 (10)	
PSQI	5 (4-8)	5 (3-6)	6 (4-9)	0.010
Poor sleep quality (n%)	52 (40)	21 (48.8)	31 (35.2)	0.13
Good sleep quality (n%)	79 (60)	22 (51.2)	57 (64.8)	
25(OH)D (ng/ml)	22 (15–35)	17.2 (11.4–28.3)	24.3 (17.5–37.4)	0.005
Deficiency (<12)	22 (18)	11 (27)	11 (13)	0.2
Insufficiency (12-30)	68 (54)	19 (46)	49 (58)	
Sufficiency (≥30)	35 (28)	11 (27)	24 (29)	

**statistically significant P-value<0.05, when variables were categorical Fisher's exact test was used and when continuous Wilcoxon rank sum test was used*

BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; NEDA-3: No Evidence of Disease Activity; WhtR: Waist-to-Height Ratio; WC: Waist Circumference; MSQoL-29: Multiple Sclerosis Quality of life 29 questionnaire; DII: Dietary Inflammatory Index; IPAQ: International Physical Activity Questionnaire; METs: Metabolic Equivalents; PA: Physical Activity; PSQI: Pittsburgh Sleep Quality Index; 25(OH)D: 25-hydroxy vitamin D

Alcohol consumption of pwMS was overall low with a median of 2 g/d, while the consumption differed significantly between men and women, with men having a higher intake (P-value=0.004). Regarding the macro- and micro-nutrients consumption 15.8% of energy intake derived from proteins, corresponding to 1 gram of protein per body kilogram, 37.4% from fats and 45.3% from carbohydrates. The median fiber consumption was low (16 g/d) compared to LARN guidelines (25 g/d) both for males and females. Only 47% of vitamin A requirements were met for the population according to the recommendations. Also, the vitamin E intake recommendations were met (65%), with women meeting significantly more the LARN guidelines (P-value=0.013). On the other hand, the median consumption of niacin (vitamin B3) was closer to the recommendation levels (83%). The median coverage of vitamin B12 coverage according to the Italian recommendations was 68%, while the difference between sexes was statistically significant with lower percentage covered by females (P-value=0.034). The median LARN % of vitamin C was 92% showing that the pwMS were almost consuming the vitamin C amount recommended. Sodium, so salt intake was above consumed above the LARN guidelines (130%). While the consumption of minerals such as potassium, calcium and magnesium was low (49, 55 and 61%, respectively. Phosphorus recommendations were met (135%), with significantly higher coverage by men compared to women (P-value=0.006). On the other hand, iron, zinc, copper, manganese and iodine needs were not met, with copper coverage being statistically significantly higher in men compared to women (P-value=0.014), while iodine median LARN % of iodine consumption was the lowest among the minerals (32%). Regarding fats

consumption the median consumption of MUFA, PUFA and cholesterol was 26, 6 g/d and 136 mg/d, respectively. Finally, a statistically significant difference was found about flavan 3-ols as part of flavonoids with women consuming significantly lower amounts than men (P-value=0.026), while isoflavones were statistically significantly higher in women than men (P-value=0.002) (Table 2).

Table 3 Nutrient intake per day of total, male and female population expressed as mean (SD) and Median (IQR)

	Total (N = 131)	Male (n=43)	Female (n=88)	P-value
Alcohol (g)	2 (0–5.8)	3.8 (0.43–9.87)	1.3 (0–4.2)	0.004
Caffeine (g)	0.13 (0.06–0.19)	0.14 (0.05–0.2)	0.1 (0.06–0.19)	0.4
Macronutrients				
Energy (Kcal)	1,647 (1,312–2,054)	1,901(1,634–2,329)	1,504 (1,259–1,913)	<0.001
Protein (g)	66 (52–83)	80.2 (60.1–94.3)	62.6 (49.2–735)	0.002
Fat (g)	66 (52–89)	69.24(52.4–91.3)	65.1 (50.6–86.9)	0.4
Carbohydrates (g)	194 (147–258)	219.5 (185.9–302)	174.2 (131.4–224.8)	<0.001
Fiber (g)	16 (13–22)	18.34(13.9–26.1)	15.8 (12.4–21.5)	0.053
Vitamins				
Vitamin A (µg)	318 (153–632)	345.5 (156–639)	311.3 (152.2–586.8)	0.8
LARN Vitamin A (%)	47 (23–85)	46.1 (20.8–85.2)	47.9 (23.4–90.3)	0.7
Vitamin K (µg)	23 (2–54)	19.3 (8.7–58.3)	25.7 (1.3–52.7)	>0.9
LARN Vitamin K (%)	17 (2–43)	14.3 (6.4–43.2)	20.5 (1–42.1)	0.9
Vitamin E (mg)	7.9 (4.9–10.4)	7.3 (4–9.9)	8.2 (6–10.9)	0.084
LARN Vitamin E (%)	65 (39–86)	56.3 (31–76.3)	68.3 (49.9–91)	0.013
Thiamin (Vitamin B1) (mg)	0.72 (0.5–1.1)	0.9 (0.6–1.2)	0.68 (0.5–0.95)	0.067
LARN Thiamin (Vitamin B1) (%)	180 (125–275)	225 (150–300)	168.8 (125–237.5)	0.067
Riboflavin (Vitamin B2) (mg)	1.12 (0.89–1.47)	1.15 (0.81–1.7)	1.12 (0.90–1.36)	0.4
LARN Riboflavin (Vitamin B2) (%)	70 (56–92)	71.9 (50.6–106.3)	69.7 (55.9–84.7)	0.4
Niacine (Vitamin B3) (mg)	15 (11–19)	16.1 (12.4–21.5)	13.7 (10–18.1)	0.042
LARN Niacine (Vitamin B3) (%)	83 (66–89)	83.1 (53.9–88.5)	83.1 (71–89)	0.4
Pantothenic acid (Vitamin B5) (mg)	2.5 (1.83–3.5)	2.7 (2–4)	2.49 (1.81–3.33)	0.2
LARN Pantothenic acid (Vitamin B5) (%)	50 (37–70)	54 (39.6–80.2)	49.8 (36.1–66.5)	0.2
Pyridoxine (Vitamin B6) (mg)	1.30 (0.93–1.77)	1.54 (0.95–2.6)	1.19 (0.93–1.66)	0.030
LARN Pyridoxine (Vitamin B6) (%)	77 (57–109)	85.6 (52.8–144.4)	74.4 (58.1–103.4)	0.3

	Total (N = 131)	Male (n=43)	Female (n=88)	P-value
Folic acid (µg)	182 (120–256)	182.1 (130–316.8)	185 (117.2–254)	0.5
LARN Folic acid (%)	55 (36–77)	55.2 (39.4–96)	56.1 (35.5–77)	0.5
Vitamin B12 (µg)	2.7 (1.3–4.15)	3.2 (1.6–5.8)	2.1 (1.3–3.9)	0.034
LARN Vitamin B12 (%)	68 (33–104)	78.8 (39.8–145)	52.5 (31.9–97.3)	0.034
Vitamin C (mg)	83 (41–133)	82.2 (41.8–167.1)	87 (40.2–131.2)	0.7
LARN Vitamin C (%)	92 (47–156)	78.3 (39.8–159.1)	102.4 (47.3–154.3)	0.4
Minerals				
Sodium (g)	1.55 (1.05–2.23)	1.89 (1.15–2.5)	1.45 (1–2.19)	0.059
LARN Sodium (%)	130 (87.7–190.7)	157.2 (95.8–208.2)	120.6 (83–182.6)	0.059
Potassium (g)	1.9 (1.57–2.49)	2.04 (1.61–2.59)	1.89 (1.56–2.41)	0.2
LARN Potassium (%)	49 (40–64)	52.3 (41.3–66.5)	48.5 (39.9–61.7)	0.2
Calcium (mg)	604 (395–880)	654 (435–1,023)	580.9 (373.1–824.6)	0.2
LARN Calcium (%)	55 (36–80)	59.5 (39.5–93)	52.8 (33.9–75)	0.2
Magnesium (mg)	215 (143–272)	240.9 (150.3–327.9)	203.3 (139.4–245.7)	0.074
LARN Magnesium (%)	61 (41–78)	68.8 (42.9–93.7)	58.1 (39.8–70.2)	0.074
Phosphorus (mg)	853 (651–1,148)	1,024 (721–1,369)	816.5 (629.3–999.6)	0.006
LARN Phosphorus (%)	135 (103–182)	162.6 (114.5–217.4)	129.6 (99.9–158.7)	0.006
Iron (mg)	7.1 (5.6–9.2)	8 (5.6–11.4)	7.1 (5.4–8.7)	0.14
LARN Iron (%)	71 (56–92)	79.8 (56.3–114)	71.3 (54.4–87)	0.14
Zinc (mg)	6.4 (4.7–9.2)	8.6 (6–10.1)	5.8 (4.7–8.2)	0.004
LARN Zinc (%)	67 (51–89)	71.7 (49.8–84.5)	64.4 (51.8–90.6)	0.8
Copper (mg)	0.76 (0.51–1.10)	0.9 (0.7–1.4)	0.70 (0.48–0.93)	0.002
LARN Copper (%)	56 (39–85)	64.3 (46.4–98.6)	53.9 (36.9–71.5)	0.014
Manganese (mg)	1.36 (0.75–2.17)	1.7 (0.8–2.6)	1.31 (0.75–1.94)	0.063
LARN Manganese (%)	58 (33–91)	68.8 (32–104)	56.7 (32.6–84.1)	0.2
Iodine (µg)	48 (28–72)	52.8 (27.4–83.1)	47.2 (30.4–66)	0.6
LARN Iodine (%)	32 (19–48)	35.2 (18.2–55.4)	31.5 (20.3–44)	0.6
Selenium (µg)	21 (12–35)	26.9 (14.2–51.3)	19.9 (10.4–29.2)	0.053
Carbohydrates				

	Total (N = 131)	Male (n=43)	Female (n=88)	P-value
Glucose (g)	7.0 (3.4–10.6)	8.3 (4.5–11.6)	5.4 (3–9.3)	0.006
Fructose (g)	9 (3–15)	13.8 (4.6–22.4)	7.2 (3.3–12.9)	0.005
Sucrose (g)	19 (9–33)	29.3 (14.6–37.7)	15.3 (7.1–26.5)	0.002
Lactose (g)	2.3 (0.4–8.6)	2.1 (0.3–9.1)	3.5 (0.57–8)	0.6
Total sugars (g)	26 (11–44)	35.5 (21.7–51.7)	21.8 (9.2–35.5)	0.009
Fats				
SFA (g)	22 (14–29)	23.2 (15.2–31.6)	20.7 (13.8–27.8)	0.089
MUFA (g)	26 (18–35)	23.6 (15.3–30.7)	26.3 (18.2–35.2)	0.4
Linolic FA (g)	5.3 (3.5–7.8)	5.3 (4.1–8.6)	5.3 (3.3–7)	0.4
Linolenic FA (g)	0.69 (0.5–1.09)	0.7 (0.5–1.1)	0.67 (0.50–1.05)	0.5
PUFA (g)	6 (4–10)	6.8 (5–11.4)	6.2 (4.1–9.2)	0.3
Cholesterol (mg)	136 (83–220)	143.9 (90–219.7)	130.4 (82.1–219)	0.5
Flavonoids				
Flavan 3-ols (mg)	66 (10–209)	156.5 (28.8–227.7)	54 (6.5–191.3)	0.026
Flavones (mg)	0.47 (0.23–1)	0.61 (0.15–1)	0.47 (0.23–0.96)	0.9
Flavonols (mg)	16 (4–41)	22.6 (8.1–59.4)	12.7 (2–35.9)	0.071
Flavonones (mg)	0.15 (0–3.07)	0.35 (0–3.8)	0.09 (0.00–3.07)	0.5
Anthocyanidins (mg)	0 (0–5)	1.4 (0–12)	0.01 (0–3.99)	0.12
Isoflavones (mg)	0 (0–0)	0 (0–0)	0 (0–0)	0.002

*statistically

signifificant

P-

value<0.05, when variables were categorical Fisher's exact test was used and when continuous Wilcoxon rank sum test was used

LARN: Livelli di Assunzione di Riferimento di Nutrienti ed Energia (Reference Intake Levels of Nutrients and Energy); SFA: Saturated Fatty Acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids

Nutrition and lifestyle habits and differences according to phase angle tertiles in pwMS

The three tertiles of phase angle defined according to the values reported were T1: <5.1°, T2: 5.1–5.6° and T3: >5.6°. Younger pwMS had significantly lower age (P-value=0.007), while the majority of male were found in the tertile with the higher phase angle, while most of the women in the lowest one (P-value=0.008). Regarding EDSS, it was statistically higher in T1 (P-value=0.008). Most of the variables related to basic characteristics and

lifestyle such as educational level, BMI, WhtR, MsQoL-29 physical and mental, smoking status and DII were not significantly different among the tertiles. On the other hand, pwMS with high PA were significantly more in T3 with higher phase angle (P-value=0.042) and participants in T3 were having significantly better sleep quality, while being the only tertile with PSQI lower than 5 (P-value=0.002). Interestingly, participants with higher phase angle had lower 25(OH)D (P-value=0.048).

Table 4 Basic characteristics, anthropometrical measurements and nutrition and lifestyle indexes for phase angle tertiles expressed as median (IQR)

Phase angle categories	T1: <5.1° (n= 46)	T2: 5.1-5.6° (n= 38)	T3: >5.6° (n= 36)	P-value
Age (years)	53.3 (43.5–60.23)	45.5 (33–55)	44.5 (32.4–51.1)	0.007
Sex (n%)				0.008
Male	13 (28)	9 (24)	20 (56)	
Female	33 (72)	29 (76)	16 (44)	
Educational level (n%)				0.3
Middle school	9 (20)	7 (18)	8 (22)	
High school	22 (48)	11 (29)	17 (47)	
Bachelor's degree	15 (33)	18 (47)	10 (28)	
Master's degree	0 (0)	2 (5.3)	1 (2.8)	
EDSS	2 (1.5–3.5)	1.5 (1–2)	1 (1–2)	0.008
≤4 (n%)	38 (83)	36 (95)	33 (92)	0.2
>4 (n%)	8 (17)	2 (5.3)	3 (8.3)	
New lesions (n%)				0.3
No	43 (93)	33 (87)	35 (97)	
Yes	3 (6.5)	5 (13)	1 (2.8)	
Growing lesions (n%)				0.5
No	46 (100)	37 (97)	35 (97)	
Yes	0 (0)	1 (2.6)	1 (2.8)	
Active lesions (n%)				0.5
No	45 (98)	36 (95)	36 (100)	

Yes	1 (2.2)	2 (5.3)	0 (0)	
NEDA3 (n%)				0.8
No	3 (6.5)	4 (1)	2 (5.6)	
Yes	43 (93)	34 (89)	34 (94)	
Weight (kg)	65.4 (57–82.4)	66.3 (56.7–79.8)	69.2 (61.3–83.2)	0.5
Height (m)	1.62 (1.57–1.71)	1.62 (1.60–1.70)	1.69 (1.61–1.76)	0.057
BMI (kg/m ²)	23.9 (21.5–27.1)	24.6 (21.1–27)	24.4 (21.1–26.5)	>0.9
Underweight (n%)	3 (6.5)	3 (7.9)	0 (0)	0.8
Normal weight (n%)	23 (50)	18 (47)	21 (58)	
Overweight (n%)	12 (26)	9 (24)	9 (25)	
Obese (n%)	8 (17)	8 (21)	6 (17)	
WhtR	0.51 (0.47–0.57)	0.50 (0.45–0.57)	0.50 (0.45–0.54)	0.6
<0.5 (n%)	18 (40)	20 (53)	17 (47)	0.5
≥0.5 (n%)	27 (60)	18 (47)	19 (53)	
WC	84.5 (77–97)	81 (73–97)	82 (77.5–93.5)	0.9
Phase angle°	4.7 (4.5–5.1)	5.5 (5.3–5.6)	6.30 (5.80–6.80)	<0.001
Smoking status (n%)				0.2
Non-smoker	26 (57)	21 (55)	18 (50)	
Smoker	7 (15)	12 (32)	12 (33)	
Ex-smoker	13 (28)	5 (13)	6 (17)	
MSQoL-29 physical	48.3 (42.5–53.4)	47.3 (41–59.2)	49 (43.6–58.8)	0.6
MSQoL-29 mental	57.4 (49.5–72.2)	59.1 (48.9–74)	65.8 (56–73.2)	0.4
DII	4.15 (2.41–4.99)	3.80 (2.85–5.13)	3.78 (2.01–4.94)	0.7
Anti-inflammatory (n%)	2 (4.3)	0 (0)	4 (11)	0.11
Pro- inflammatory (n%)	44 (96)	38 (100)	32 (89)	
Medi-Lite	10.5 (9–12)	10 (8–11)	11 (9–12)	0.4
Low adherence (n%)	4 (8.7)	11 (29)	8 (22)	0.055
High adherence (n%)	42 (91)	27 (71)	28 (78)	
IPAQ (METs)	587 (219–1,560)	570 (306–1,040)	1,052 (231–2,750)	0.14
Low PA (n%)	26 (57)	24 (63)	14 (39)	0.042
Moderate PA (n%)	17 (37)	10 (26)	12 (33)	

High PA (n%)	3 (6.5)	4 (11)	10 (28)	
PSQI	6 (4–8)	6.5 (5–9)	4 (3–6)	0.002
Poor sleep quality (n%)	29 (63)	29 (76)	14 (39)	0.004
Good sleep quality (n%)	17 (37)	9 (24)	22 (61)	
25(OH)D (ng/ml)	25.2 (18.1–39.1)	22.1 (12.3–29.1)	18.2 (13–28.2)	0.048
Deficiency (<12)	6 (13)	8 (22)	7 (21)	0.3
Insufficiency (12–30)	21 (47)	20 (56)	19 (58)	
Sufficiency (≥30)	18 (40)	8 (22)	7 (21)	

*statistically significant P -value<0.05, when variables were categorical Fisher's exact test and Pearson's Chi-squared test were used and when continuous Wilcoxon rank sum test was used

BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; NEDA-3: No Evidence of Disease Activity; WhtR: Waist-to-Height Ratio; WC: Waist Circumference; MSQoL-29: Multiple Sclerosis Quality of life 29 questionnaire; DII: Dietary Inflammatory Index; IPAQ: International Physical Activity Questionnaire; METs: Metabolic Equivalents; PA: Physical Activity; PSQI: Pittsburgh Sleep Quality Index; 25(OH)D: 25-hydroxy vitamin D

Regarding nutrient composition of the diets of pwMS according to phase angle tertiles, no significant differences were found for alcohol, caffeine, vitamin A, vitamin K, vitamin E, vitamin B2, niacin, vitamin B6 and vitamin C. The median thiamin (vitamin B1) consumption was statistically higher in T3 compared to the other two tertiles, with the lowest LARN % reported for T2 (P -value=0.042). Similarly, for vitamin B5, folic acid and vitamin B12, the intake of these vitamins was statistically higher in T3 with the lowest LARN % reported for T2 (P -values=0.029, 0.033, 0.013, respectively). In respect of mineral intake per phase angle tertile, no statistically significant differences were found for sodium, potassium, calcium, magnesium, iron, copper, manganese and selenium. For phosphorus and iodine, the statistically significant higher LARN % was found for T3 with T2 having the lowest intake (P -value 0.001 and 0.01, respectively), while zinc intake increased by tertile with T3 having statistically higher LARN % compared to the other two tertiles (P -value=0.027). For flavonones and anthocyanins, statistically higher intake was noted for T3 (P -value=0.022 and <0.001, respectively) (Table 4).

Table 5 Nutrient intake per day of pwMS per phase angle tertile expressed as Median (IQR)

Phase angle categories	T1: <5.1 (n= 46)	T2: 5.1-5.6 (n= 38)	T3: >5.6 (n= 36)	P-value
Alcohol (g)	0.83 (0–3.7)	2.1 (0–6.2)	3.8 (0.5–7.4)	0.080

<i>Phase angle categories</i>	T1: <5.1 (n= 46)	T2: 5.1-5.6 (n= 38)	T3: >5.6 (n= 36)	P-value
Caffeine (g)	0.12 (0.04–0.19)	0.13 (0.06–0.2)	0.13 (0.06–0.19)	0.5
Macronutrients				
Energy (Kcal)	1,552 (1,300–1,829)	1,669 (1,380–2,013)	1,880 (1,418–2,153)	0.14
Protein (g)	63.7 (48.9–75.1)	62.1 (47.1–71.5)	74.4 (61.1–104.8)	0.003
Fat (g)	59.8 (44–91.3)	68.6 (55.9–84.4)	67.1 (55.3–92.9)	0.4
Carbohydrates (g)	186.59 (145.19–224.24)	197.7 (147.1–257.6)	198.3 (149.3–276.2)	0.6
Fiber (g)	17 (13.8–21.5)	15.8 (12.7–19.3)	16.9 (11.7–25)	0.6
Vitamins				
Vitamin A (µg)	268.2 (145.5–639)	300.3 (113.2–542)	381.5 (227–611.1)	0.4
LARN Vitamin A (%)	39.1 (22.4–85.2)	46.2 (17.3–82.5)	54.9 (33.6–86.1)	0.4
Vitamin K (µg)	22.4 (4.7–53.4)	37 (2.4–61.1)	16.3 (5.2–56)	0.6
LARN Vitamin K (%)	17.9 (3.5–41.4)	29.6 (1.8–48.9)	13.1 (3.9–43)	0.5
Vitamin E (mg)	7.9 (5.4–9)	8.2 (4.7–10.4)	8.2 (5.3–11.4)	0.7
LARN Vitamin E (%)	64.4 (43.2–74.3)	67.5 (39.2–86.9)	63.7 (41.9–93.7)	0.9
Thiamin (Vitamin B1) (mg)	0.7 (0.5–0.88)	0.65 (0.44–0.9)	0.95 (0.62–1.35)	0.042
LARN Thiamin (Vitamin B1) (%)	175 (125–220)	162.5 (110–225)	237.5 (155–336.3)	0.042
Riboflavin (Vitamin B2) (mg)	1.13 (0.8–1.35)	1.05 (0.9–1.32)	1.38 (0.94–1.72)	0.075
LARN Riboflavin (Vitamin B2) (%)	70.3 (50–84.4)	65.6 (56.3–82.5)	86.3 (58.8–107.5)	0.075
Niacine (Vitamin B3) (mg)	14.1 (10.7–19.2)	14.7 (11–18.1)	15.1 (10.7–21.2)	0.5
LARN Niacine (Vitamin B3) (%)	81.7 (61.5–87.7)	83.1 (64.3–90.2)	84 (64.8–88.6)	0.8
Pantothenic acid (Vitamin B5) (mg)	2.71 (2.01–3.49)	2.13 (1.74–2.89)	2.93 (2.01–4.05)	0.029

Phase angle categories	T1: <5.1 (n= 46)	T2: 5.1-5.6 (n= 38)	T3: >5.6 (n= 36)	P-value
LARN Pantothenic acid (Vitamin B5) (%)	54.2 (40.2–69.8)	42.5 (34.8–57.8)	58.5 (40.2–80.9)	0.029
Pyridoxine (Vitamin B6) (mg)	1.24 (0.93–1.84)	1.19 (0.94–1.5)	1.51 (0.99–2.22)	0.094
LARN Pyridoxine (Vitamin B6) (%)	77.2 (56.3–115)	71.3 (58.1–88.9)	86.8 (58.3–129.4)	0.12
Folic acid (µg)	202.7 (120.4–252.2)	153.3 (112.6–198.2)	225.3 (139.9–323.3)	0.033
LARN Folic acid (%)	61.4 (36.5–76.4)	46.4 (34.1–60.1)	68.3 (42.4–98)	0.033
Vitamin B12 (µg)	2.6 (1.25–3.33)	1.78 (0.9–4.5)	3.74 (2.19–6.01)	0.013
LARN Vitamin B12 (%)	64.9 (31.3–83.3)	44.4 (22.5–112.5)	93.4 (54.6–150.1)	0.013
Vitamin C (mg)	99.8 (47.4–160.8)	71.9 (37.8–101.8)	89.8 (50.6–159.8)	0.2
LARN Vitamin C (%)	113.1 (52.3–185.6)	75.1 (43.7–119.8)	96.9 (51.3–187)	0.3
Minerals				
Sodium (g)	1.55 (0.98–2.15)	1.38 (1–2.1)	1.84 (1.13–2.54)	0.2
LARN Sodium (%)	129 (81.5–179.4)	114.7 (83.1–175.2)	153.3 (93.8–211.4)	0.2
Potassium (g)	2.03 (1.56–2.49)	1.81 (1.59–2.14)	2.22 (1.65–2.72)	0.2
LARN Potassium (%)	52.1 (40.1–63.9)	46.3 (40.8–54.8)	56.9 (42.3–69.7)	0.2
Calcium (mg)	619.2 (291.6–811.5)	475.6 (348.8–807.6)	634.4 (462.8–1,093.2)	0.077
LARN Calcium (%)	56.3 (26.5–73.8)	43.2 (31.7–73.4)	57.7 (42.1–99.4)	0.077
Magnesium (mg)	208.7 (136.3–267)	198.7 (149–239)	219.2 (156.6–339.7)	0.2
LARN Magnesium (%)	59.6 (39–76.3)	56.8 (42.6–68.3)	62.6 (44.8–97.1)	0.2
Phosphorus (mg)	839.9 (622.2–1,052.6)	712.4 (601.9–971.6)	1,040.5 (819.4–1,405.4)	0.001
LARN Phosphorus (%)	133.3 (98.8–167.1)	113.1 (95.5–154.2)	165.15 (130.1–223.1)	0.001
Iron (mg)	7.1 (5.6–8.7)	7.49 (5.74–8.91)	7.20 (5.8–10.9)	0.5
LARN Iron (%)	71 (55.8–87.4)	74.9 (57.4–89.1)	72 (58.3–109.2)	0.5
Zinc (mg)	5.8 (4.7–7.7)	6.1 (4.5–8.7)	8.4 (6–10.1)	0.003
LARN Zinc (%)	61.8 (47.9–76.9)	62.7 (48.9–88.9)	75.4 (60.7–96.9)	0.027
Copper (mg)	0.78 (0.51–1)	0.74 (0.52–1)	0.79 (0.53–1.34)	0.4

Phase angle categories	T1: <5.1 (n= 46)	T2: 5.1-5.6 (n= 38)	T3: >5.6 (n= 36)	P-value
LARN Copper (%)	59.6 (39.2–72.1)	56.7 (40–73.9)	58.2 (38.9–99.3)	0.4
Manganese (mg)	1.54 (0.95–2.35)	1.31 (0.9–2)	1.13 (0.68–2.62)	0.7
LARN Manganese (%)	64.2 (41.3–94.4)	56.7 (38.8–87)	47.8 (27.2–104.8)	0.7
Iodine (µg)	48.1 (27.9–77.6)	33.6 (16.9–55.9)	56.2 (41.7–78.8)	0.010
LARN Iodine (%)	32.1 (18.6–51.7)	22.4 (11.3–37.3)	37.4 (27.8–52.6)	0.010
Selenium (µg)	20.1 (10.6–28.6)	18.9 (9–32.3)	22 (13.8–49.2)	0.3
Carbohydrates				
Glucose (g)	8.2 (4–10.6)	7 (3.2–11)	6.6 (2.7–10.7)	0.5
Fructose (g)	9.9 (4.5–17.5)	9.3 (2.4–14.6)	7.3 (2.5–13.6)	0.2
Sucrose (g)	17.9 (9–32.2)	20.8 (10.5–33.9)	20.8 (7.3–32.4)	>0.9
Lactose (g)	3.1 (0.7–8.1)	1.05 (0.21–7.4)	6.4 (0.33–9.9)	0.4
Total sugars (g)	26.4 (13.3–35.5)	36.4 (16.9–48)	23.3 (7.4–41.5)	0.3
Fats				
SFA (g)	18.9 (12.6–32.9)	22.5 (15.2–28.7)	22.4 (16.7–28.9)	0.5
MUFA (g)	23.4 (18.2–31.9)	28.9 (14.9–34.9)	26 (18.3–33.2)	0.8
Linolic FA (g)	4.9 (3.5–6.8)	5.5 (3.3–7.8)	5.8 (3.9–8.6)	0.5
Linolenic FA (g)	0.67 (0.5–1.08)	0.61 (0.46–0.9)	0.74 (0.6–1.1)	0.4
PUFA (g)	5.8 (4.4–9.1)	6.2 (4.1–8.7)	7.7 (5–10.8)	0.4
Cholesterol (mg)	134.4 (79.4–226.8)	116.1 (60.8–194.9)	145.9 (98.3–318.9)	0.077
Flavonoids				
Flavan-3 ols	54 (6.7–205.9)	67.1 (6.7–315)	97.8 (28–200)	0.8
Flavones	0.58 (0.31–1.41)	0.46 (0.17–1.04)	0.48 (0.24–0.88)	0.3
Flavonols	13.8 (4–42.3)	23.7 (2–79.1)	17.3 (6.6–33.8)	0.7
Flavonones	0.05 (0–3.07)	0.05 (0–2.31)	2.01 (0.15–4.63)	0.022
Anthocyanins	0 (0–4)	0 (0–2.96)	3.99 (0.7–16.6)	<0.001
Isoflavones	0 (0–0)	0 (0–0)	0 (0–0)	0.053

**statistically significant P-value<0.05, when variables were categorical Fisher's exact test and Pearson's Chi-squared test were used and when continuous Wilcoxon rank sum test was used*

LARN: Livelli di Assunzione di Riferimento di Nutrienti ed Energia (Reference Intake Levels of Nutrients and Energy); SFA: Saturated Fatty Acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids

Nutrition and lifestyle habits and differences according to DII tertiles in pwMS

The tertiles created for DII according to the available data were the followings: T1: <2.93, T2: 2.93-4.79 and T3: >4.79, showing that the majority of the participants had a pro-inflammatory diet. Most of the various characteristics of the population, anthropometric measurements and indexes, such as age, sex, education did not differ significantly among the tertiles. Medi-Lite score was statistically higher when DII was lower, which can be explained, since the Mediterranean diet is defined as an anti-inflammatory diet according to the amount of food groups suggested (P-value<0.001) (Table 5).

Table 6 Basic characteristics, anthropometrical measurements and nutrition and lifestyle indexes for the DII tertiles expressed as mean (SD) and median (IQR)

DII tertiles	T1: <2.93 (n= 44)	T2: 2.93-4.79 (n= 43)	T3: >4.79 (n= 44)	P-value
Age (years)	45.6 (31.9–59.1)	47.5 (39.5–57.5)	44.8 (34.9–54.2)	0.7
Sex (n%)				0.4
Female	26 (59)	30 (70)	32 (73)	
Male	18 (41)	13 (30)	12 (27)	
Educational level (n%)				0.2
Middle school	6 (14)	8 (19)	11 (25)	
High school	18 (41)	20 (47)	17 (39)	
Bachelor's degree	20 (45)	13 (30)	12 (27)	
Master's degree	0 (0)	2 (4.7)	4 (9.1)	
EDSS	1.5 (1–2)	1.5 (1–2.5)	1.5 (1–2.75)	0.4
<4 (n%)	42 (95)	37 (86)	38 (86)	0.4
>4 (n%)	2 (4.5)	6 (14)	6 (14)	
New lesions (n%)				0.10
No	43 (98)	37 (86)	42 (95)	

Yes	1 (2.3)	6 (14)	2 (4.5)	
Growing lesions (n%)				0.5
No	44 (100)	42 (98)	42 (95)	
Yes	0 (0)	1 (2.3)	2 (4.5)	
Active lesions (n%)				0.034
No	44 (100)	40 (93)	44 (100)	
Yes	0 (0)	3 (7)	0 (0)	
NEDA3 (n%)				0.2
No	1 (2.3)	5 (12)	4 (9.1)	
Yes	43 (98)	38 (88)	40 (91)	
Weight (kg)	64 (58.6–74.7)	68.7 (56.7–85.3)	63.8 (54–81.6)	0.5
Height (m)	1.66 (1.6–1.72)	1.6 (1.6–1.7)	1.64 (1.59–1.73)	0.9
BMI (kg/m ²)	23.3 (21.1–25.7)	24.7 (21.9–28)	23.8 (20.5–26.6)	0.3
Underweight (n%)	1 (2.3)	3 (7)	5 (11)	0.4
Normal weight (n%)	29 (66)	20 (47)	20 (45)	
Overweight (n%)	7 (16)	12 (28)	12 (27)	
Obese (n%)	7 (16)	8 (19)	7 (16)	
WhtR	0.5 (0.44–0.54)	0.51 (0.46–0.56)	0.50 (0.45–0.58)	0.5
<0.5 (n%)	20 (49)	17 (40)	21 (53)	0.5
≥0.5 (n%)	21 (51)	25 (60)	19 (48)	
WC	82 (77–88)	86(76–97)	81.3 (72.5–100)	0.6
Phase angle°	5.5 (5–5.8)	5.3 (4.9–5.8)	5.3 (4.9–5.7)	0.9
Smoking status (n%)				0.4
Non-smoker	26 (59)	23 (53)	20 (45)	
Smoker	7 (16)	12 (28)	15 (34)	
Ex-smoker	11 (25)	8 (19)	9 (20)	
MSQoL-29 physical	51.6 (42.9–59.2)	48.5 (42.1–57.3)	48.1 (41.6–59.7)	>0.9
MSQoL-29 mental	61.2 (50.6–71.7)	58.9 (49.9–74)	60 (49.4–73.4)	>0.9
DII	1.68 (0.5–2.4)	4.1 (3.6–4.4)	5.5 (5.1–6)	<0.001
Anti-inflammatory (n%)	7 (16)	0 (0)	0 (0)	<0.001

Pro- inflammatory (n%)	37 (8)	43 (100)	44 (100)	
Medi-Lite	11.5 (10–13)	11 (9–12)	9.5 (8–11)	<0.001
Low adherence (n%)	2 (4.5)	10 (23)	13 (30)	0.008
High adherence (n%)	42 (95)	33 (77)	31 (70)	
IPAQ (METs)	834 (347–1,922)	558 (198–1,547)	686 (182–1,713)	0.5
Low PA (n%)	20 (45)	24 (56)	25 (57)	0.5
Moderate PA (n%)	14 (32)	14 (33)	14 (32)	
High PA (n%)	10 (23)	5 (12)	5 (11)	
PSQI	4.5 (3–7)	5 (3–8)	6 (4–8)	0.087
Poor sleep quality (n%)	22 (50)	25 (58)	32 (73)	
Good sleep quality (n%)	22 (50)	18 (42)	12 (27)	
25(OH)D (ng/ml)	22.4 (17.1–35.4)	19.3 (13–29.1)	23.4 (14.1–34.6)	0.4
Deficiency (<12)	5 (12)	9 (21)	8 (20)	0.7
Insufficiency (12–30)	23 (55)	24 (57)	21 (51)	
Sufficiency (≥30)	14 (33)	9 (21)	12 (29)	

**statistically significant P-value<0.05, when variables were categorical Fisher's exact test and Pearson's Chi-squared test were used and when continuous Wilcoxon rank sum test was used*

BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; NEDA-3: No Evidence of Disease Activity; WhtR: Waist-to-Height Ratio; WC: Waist Circumference; MSQoL-29: Multiple Sclerosis Quality of life 29 questionnaire; DII: Dietary Inflammatory Index; IPAQ: International Physical Activity Questionnaire; METs: Metabolic Equivalents; PA: Physical Activity; PSQI: Pittsburgh Sleep Quality Index; 25(OH)D: 25-hydroxy vitamin D

For the calculation of DII multiple nutrients have been used, for this reason multiple of them show to have statistically significant differences among the tertiles, showing that the higher consumption of healthy pro-inflammatory nutrients such as fiber, vitamin A, vitamin E, thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), folic acid, vitamin C, magnesium, iron, zinc, healthy fatty acids and some flavonoids are related to lower DII. Other micronutrients not included in the calculation of DII (pantothenic acid, potassium, phosphorus, copper and manganese) showed that their

higher consumption based on the tertiles resulted with statistically significantly lower DII (P-value=0.001, <0.001, 0.005, 0.005 and 0.002, respectively) (Table 6).

Table 7 Nutrient intake per day of pwMS per DII tertile expressed as mean (SD) and Median (IQR)

DII tertiles	T1: <2.93 (n= 44)	T2: 2.93-4.79 (n= 43)	T3: >4.79 (n= 44)	P-value
Alcohol (g)	2.66 (0.43–7.11)	1.23 (0.00–3.97)	1.68 (0.00–4.31)	0.2
Caffeine (g)	0.13 (0.05–0.19)	0.13 (0.06–0.21)	0.10 (0.06–0.19)	0.8
Macronutrients				
Energy (Kcal)	1,673 (1,329–2,120)	1,6467 (1,300–2,054)	1,634 (1,255–1,949)	0.5
Protein (g)	69.1 (58.2–88.3)	67.70(47.1–82.6)	60.5 (47–81)	0.090
Fat (g)	67.2 (47.4–89.3)	66.5 (55.9–92.5)	64.9 (51–75.1)	0.5
Carbohydrates (g)	203 (155.3–282.8)	180.6 (145.2–216.2)	191.9 (132.3–250.9)	0.3
Fiber (g)	24.3 (16.7–30.3)	16.3 (13.8–19.6)	12.4 (9.2–16.3)	<0.001
Vitamins				
Vitamin A (µg)	496.3 (181.2–895.2)	291.5 (186.4–536.1)	231.2 (122.1–466.5)	0.037
LARN Vitamin A (%)	73.2 (24.7–130.3)	44.5 (26.2–79.4)	34.2 (18.9–68.9)	0.043
Vitamin K (µg)	26.5 (9.4–64.1)	17.4 (1.9–55.6)	21.4 (0.75–50.5)	0.4
LARN Vitamin K (%)	21.2 (7–47.5)	14 (1.5–43.6)	17.1 (0.59–39.5)	0.4
Vitamin E (mg)	10.3 (8.4–14)	7.4 (5.2–9.3)	6 (3.6–8.5)	<0.001
LARN Vitamin E (%)	82.8 (66.4–110.9)	61.5 (41.5–73.5)	50.3 (29.8–68.9)	<0.001
Thiamin (Vitamin B1) (mg)	1.01 (0.75–1.36)	0.67 (0.59–0.95)	0.48 (0.40–0.72)	<0.001
LARN Thiamin (Vitamin B1) (%)	252.5 (186.3–338.8)	167.5 (147.5–237.5)	118.8 (98.8–180)	<0.001
Riboflavin (Vitamin B2) (mg)	1.4 (1.1–1.7)	1.1 (0.8–1.5)	0.95 (0.72–1.20)	<0.001
LARN Riboflavin (Vitamin B2) (%)	84.4 (68.8–106.3)	68.8 (50–93.8)	59.4 (44.7–74.7)	<0.001
Niacin (Vitamin B3) (mg)	17.2 (13.8–21.2)	13.8 (10.6–19.1)	11.6 (8.4–16.7)	<0.001
LARN Niacin (Vitamin B3) (%)	82.9 (69.2–88.7)	84.13 (74.4–89.1)	78.9 (52.3–88.2)	0.3

<i>DII tertiles</i>	T1: <2.93 (n= 44)	T2: 2.93-4.79 (n= 43)	T3: >4.79 (n= 44)	P-value
Pantothenic acid (Vitamin B5) (mg)	3 (2.1–3.9)	2.4 (2.1–3.6)	1.9 (1.4–2.9)	0.001
LARN Pantothenic acid (Vitamin B5) (%)	59.6 (42.2–7)	49.2 (42–71)	38.3 (28.8–57.4)	0.001
Pyridoxine (Vitamin B6) (mg)	1.8 (1.3–2.3)	1.32 (1–1.7)	0.93 (0.63–1.3)	<0.001
LARN Pyridoxine (Vitamin B6) (%)	111.3 (77.1–143.1)	81.3 (62.5–102.5)	57.1 (38.8–77.2)	<0.001
Folic acid (µg)	293.9 (215.8–414)	179.5 (129.6–249.4)	117.2 (92.9–165.1)	<0.001
LARN Folic acid (%)	89.1 (65.4–125.5)	54.4 (39.3–75.6)	35.5 (28.1–50)	<0.001
Vitamin B12 (µg)	3 (1.3–5)	2.5 (1.2–3.9)	2.4 (1.4–4.3)	0.7
LARN Vitamin B12 (%)	74.4 (32.6–124.1)	62.50(29.5–97.8)	60.5 (35.5–108.1)	0.7
Vitamin C (mg)	131.8 (95.5–215)	88.4 (50.1–141.3)	39.1 (23.7–67.7)	<0.001
LARN Vitamin C (%)	143.4 (108–238)	101.4 (53.4–159.1)	46 (25.6–73.3)	<0.001
Minerals				
Sodium (g)	1.35 (0.86–2.27)	1.56 (1.12–2.26)	1.6 (1.1–2.4)	0.5
LARN Sodium (%)	112 (71.9–189.1)	130.1 (93.1–188.2)	135.4 (89.8–199.8)	0.5
Potassium (g)	2.56 (2.24–3.22)	1.88 (1.64–2.37)	1.5 (1.18–1.69)	<0.001
LARN Potassium (%)	65.6 (57.5–82.6)	48.2 (42–60.7)	38.5 (30.3–43.4)	<0.001
Calcium (mg)	645.5 (514.9–985.2)	558.9 (297.4–877)	588.3 (348.4–809.8)	0.2
LARN Calcium (%)	58.7 (46.8–89.6)	50.8 (27–79.7)	53.5 (31.7–73.6)	0.2
Magnesium (mg)	267.2 (173.7–327)	209.9 (169–262.4)	156.6 (120.1–225.2)	<0.001
LARN Magnesium (%)	76.3 (49.6–93.4)	60 (48.3–75)	44.8 (34.3–64.3)	<0.001
Phosphorus (mg)	946 (808–1,314)	816 (655–1,167)	710 (543.5–961.4)	0.005
LARN Phosphorus (%)	150.2 (128.2–208.6)	129.5 (104–185.2)	112.7 (86.3–152.6)	0.005
Iron (mg)	9.5 (8–12.2)	6.5 (5.5–8.7)	6.1 (4.1–7.1)	<0.001
LARN Iron (%)	95.1 (79.5–121.6)	65 (55–87)	61.1 (40.5–70.5)	<0.001
Zinc (mg)	7.7 (5.2–10.6)	6.4 (5.4–9.1)	5.5 (4–7.9)	0.016
LARN Zinc (%)	75.4 (52.7–109.1)	67.8 (51.7–96)	59.9 (44.6–76)	0.051

DII tertiles	T1: <2.93 (n= 44)	T2: 2.93-4.79 (n= 43)	T3: >4.79 (n= 44)	P-value
Copper (mg)	0.92 (0.67–1.42)	0.79 (0.48–1.00)	0.60 (0.48–0.81)	0.005
LARN Copper (%)	70.7 (48.6–101.8)	60 (36.9–72.3)	45.8 (35.9–62.3)	0.004
Manganese (mg)	1.8 (1.1–2.7)	1.2 (0.7–1.9)	1.1 (0.6–1.8)	0.002
LARN Manganese (%)	72 (46–116.1)	48.8 (26.4–82.6)	46.7 (24.8–74.6)	0.002
Iodine (µg)	52.1 (32.3–75)	40.7 (27.9–75.3)	48 (32.1–71.3)	0.6
LARN Iodine (%)	34.7 (21.5–50)	27.1 (18.6–50.2)	32 (21.4–47.5)	0.6
Selenium (µg)	25.6 (13.6–50)	20.1 (12.3–26.7)	19.4 (10.6–35.4)	0.13
Carbohydrates				
Glucose (g)	9.7 (6–12.5)	7.2 (3.7–10.7)	3.9 (1.3–7)	<0.001
Fructose (g)	13.6 (6.3–18.7)	7.9 (3.5–15.4)	5 (1.4–11.5)	<0.001
Sucrose (g)	22.4 (11–37.6)	18.7 (8.6–37.5)	17.3 (5.2–28.4)	0.078
Lactose (g)	2.03 (0.27–7.5)	5.3 (0.4–10)	3.7 (0.1–8.3)	0.4
Total sugars (g)	19.3 (7.4–38.7)	26.4 (17.4–43.6)	32 (13–46.6)	0.2
Fats				
SFA (g)	19.5 (11.7–24.5)	23.8 (15.4–33.5)	22.6 (15.1–28.6)	0.073
MUFA (g)	27.6 (19.1–39.8)	26.3 (18–36.4)	21.9 (15–30.4)	0.13
Linolic FA (g)	6.7 (3.9–10.9)	5.2 (3.5–6.5)	4.6 (3–6.3)	0.026
Linolenic FA (g)	0.89 (0.59–1.40)	0.72 (0.51–1.10)	0.56 (0.44–0.82)	<0.001
PUFA (g)	7.5 (5–13.4)	6.3 (4.4–9.7)	5.4 (3.7–8.4)	0.018
Cholesterol (g)	107.8 (66.1–271.2)	145.6 (111.5–213.6)	128.8 (86.7–219)	0.4
Flavonoids				
Flavan-3 ols (mg)	184.7 (27.5–333)	58.9 (6.7–188.8)	42.2 (6.3–169.8)	0.015
Flavones (mg)	0.72 (0.39–1.67)	0.50 (0.31–1.18)	0.26 (0.02–0.53)	<0.001
Flavonols (mg)	29.6 (12.5–79.9)	16.61 (5.50–45.89)	4.64 (0.93–17.11)	<0.001
Flavonones (mg)	0.49 (0.04–3.46)	0.07 (0.00–2.88)	0.05 (0.00–3.84)	0.2
Anthocyanins (mg)	0.02 (0–8.81)	1.37 (0–9.8)	0 (0–2.03)	0.050
Isoflavones (mg)	0 (0–0)	0 (0–0)	0 (0–0)	0.11

*statistically significant *P*-value<0.05, when variables were categorical Fisher's exact test and Pearson's Chi-squared test were used and when continuous Wilcoxon rank sum test was used

LARN: Livelli di Assunzione di Riferimento di Nutrienti ed Energia (Reference Intake Levels of Nutrients and Energy); SFA: Saturated Fatty Acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids

Correlations among anthropometrical measurements, nutrition and lifestyle indexes and nutrient intake

Spearman's rho test showed statistically significant correlations between anthropometrical measurements, nutrition and lifestyle indexes and nutrient intake. Higher alcohol consumption showed a weak negative correlation with EDSS ($p=-0.24$; $p\text{-value}=0.0054$) (Table 7).

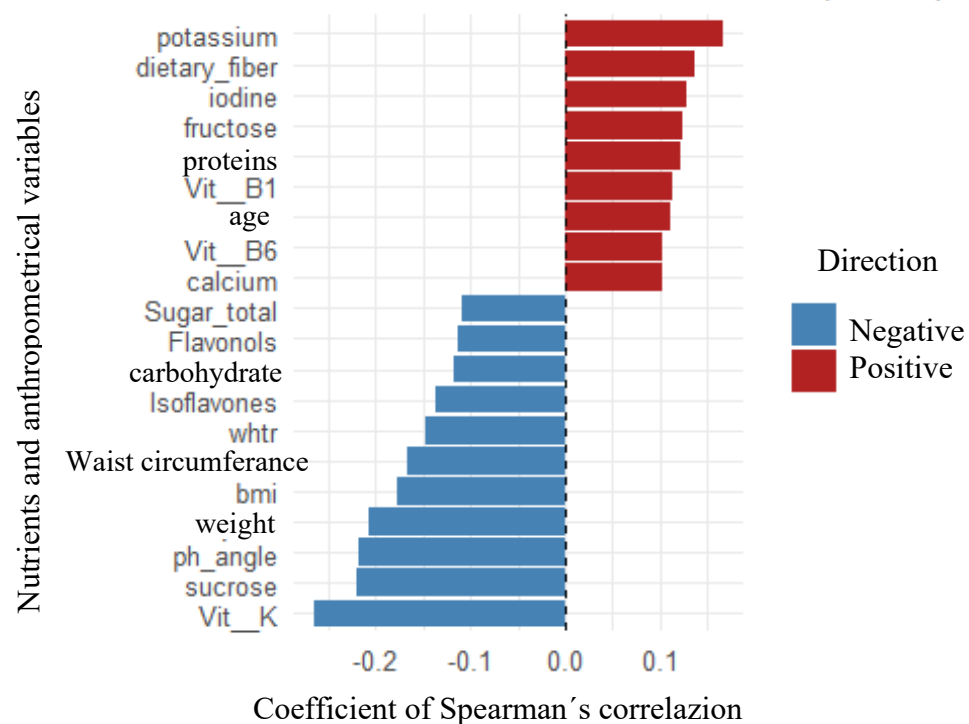


Figure 3 Correlations of 25(OH)D with nutrients and anthropometrical measurements

Caffeine was weakly positively correlated with WhtR ($p=0.21$; $p\text{-value}=0.019$). Interestingly, higher energy and protein consumption were weakly correlated with better sleep quality ($p=-0.2$; $p\text{-value}=0.024$ and $p=-0.33$; $p\text{-value}=0.00012$), while higher carbohydrates consumption was weakly correlated with better, so lower EDSS ($p=-0.21$; $p\text{-value}=0.018$). Moreover, higher fiber consumption was weakly correlated with lower WhtR ($p=0.23$; $p\text{-value}=0.0096$) and higher adherence to the Mediterranean diet ($p=0.3$; $p\text{-value}=0.00055$). Other interesting findings about EDSS showed that higher lactose, flavan-3 ols and phase angle were weakly correlated with lower EDSS, while higher EDSS was weakly correlated with lower sleep quality and both physical and mental quality of life. Medi-Lite was positively correlated with multiple micronutrients that can be found in the main food groups of the Mediterranean diet, such as vitamin A, R, C, folic acid, potassium, calcium, manganese and linolenic fatty acids, while it was negatively correlated with lactose, total sugars and SFA. The DII was negatively correlated with most of the nutrients that are used for its calculation such as vitamin A, E, B6 etc. Higher BMI was weakly correlated with lower linolic FA and PUFA and higher WhtR was correlated with higher age, but lower vitamin E, linolic FA and PUFA intake. On the other hand, phase angle was weakly correlated with higher vitamin B12, phosphorus, zinc intake and sleep quality, but with lower age and 25(OH)D. Additionally, higher physical activity was weakly correlated with better sleep quality and mental quality of life. Better sleep quality was weakly correlated with higher energy, protein, vitamin B2, B3, B6, sodium, potassium, phosphorus, zinc intake and mental quality of life. As regards MSQoL higher physical MSQoL was weakly correlated with lower vitamin B3 intake, but higher calcium intake and moderately correlated with better mental MSQoL. Furthermore, higher mental MSQoL was weakly correlated with higher vitamin K and copper intake, but lower vitamin E and B3 intake. Finally, higher 25(OH)D was weakly correlated with lower BMI, vitamin K and sucrose intake (Table 7, Figure 3).

Table 8 Spearman's rho correlations among anthropometric measurements, nutrition and lifestyle indexes and nutrient intake expressed as ρ coefficient (P-value)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
Alcohol (g)	0.02 (p=0.86)	-0.24 (p=0.0054)	0.13 (p=0.14)	0.21 (p=0.014)	0.01 (p=0.9)	0.03 (p=0.73)	0.11 (p=0.24)	0.17 (p=0.07)	0.03 (p=0.75)	-0.11 (p=0.2)	0.1 (p=0.27)	-0.13 (p=0.12)	0.13 (p=0.15)	0.15 (p=0.091)	0 (p=0.99)
Caffeine (g)	0.07 (p=0.43)	-0.07 (p=0.41)	0.15 (p=0.095)	-0.03 (p=0.76)	0.16 (p=0.075)	0.21 (p=0.019)	0.23 (p=0.011)	0.04 (p=0.63)	-0.1 (p=0.24)	-0.02 (p=0.8)	-0.1 (p=0.26)	-0.04 (p=0.68)	-0.02 (p=0.79)	-0.14 (p=0.099)	-0.07 (p=0.44)
Macronutrients															
Energy (Kcal)	-0.1 (p=0.26)	-0.21 (p=0.014)	0.02 (p=0.78)	0.39 (p=0.0039)	-0.18 (p=0.045)	-0.18 (p=0.042)	-0.04 (p=0.69)	0.14 (p=0.12)	-0.07 (p=0.43)	-0.1 (p=0.25)	0.1 (p=0.25)	-0.2 (p=0.024)	0.07 (p=0.4)	0.16 (p=0.063)	0 (p=0.97)
Protein (g)	-0.22 (p=0.013)	-0.16 (p=0.06)	0.03 (p=0.75)	0.33 (p=0.0012)	-0.13 (p=0.14)	-0.16 (p=0.073)	-0.05 (p=0.61)	0.25 (p=0.0068)	-0.04 (p=0.62)	-0.22 (p=0.013)	0.11 (p=0.21)	-0.33 (p=0.0012)	0.02 (p=0.79)	0.14 (p=0.12)	0.02 (p=0.83)
Fat (g)	-0.13 (p=0.13)	-0.13 (p=0.13)	-0.06 (p=0.51)	0.17 (p=0.05)	-0.15 (p=0.087)	-0.19 (p=0.039)	-0.12 (p=0.2)	0.09 (p=0.31)	-0.04 (p=0.63)	-0.06 (p=0.51)	0.06 (p=0.46)	-0.1 (p=0.24)	0 (p=0.96)	0.04 (p=0.67)	0.04 (p=0.66)
Carbohydrates (g)	-0.07 (p=0.42)	-0.21 (p=0.018)	0.03 (p=0.7)	0.36 (p=0.024)	-0.14 (p=0.11)	-0.13 (p=0.06)	0 (p=0.96)	0.04 (p=0.64)	-0.06 (p=0.23)	-0.06 (p=0.48)	0.06 (p=0.52)	-0.12 (p=0.19)	0.06 (p=0.47)	0.19 (p=0.032)	-0.06 (p=0.5)
Fiber (g)	-0.01 (p=0.89)	-0.11 (p=0.21)	-0.06 (p=0.46)	0.17 (p=0.055)	-0.16 (p=0.071)	-0.23 (p=0.0096)	-0.17 (p=0.058)	0.02 (p=0.84)	0.3 (p=0.00055)	-0.63 (p=0.0066)	0.11 (p=0.19)	-0.19 (p=0.026)	0.18 (p=0.038)	0.1 (p=0.27)	0.14 (p=0.13)
Vitamins															
Vitamin A (µg)	0.01 (p=0.89)	-0.03 (p=0.73)	-0.02 (p=0.86)	0.08 (p=0.36)	-0.03 (p=0.7)	-0.06 (p=0.48)	-0.06 (p=0.5)	0.07 (p=0.45)	0.22 (p=0.014)	-0.26 (p=0.0027)	0.11 (p=0.2)	-0.01 (p=0.9)	0.03 (p=0.74)	0.06 (p=0.52)	0.05 (p=0.61)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
LARN Vitamin A (%)				0.04	-0.04	-0.07	-0.08	0.06							
		-0.03	-0.04	(p=0.68)	(p=0.66)	(p=0.42)	(p=0.35)	(p=0.52)	0.22	(p=0.0033)	0.14	0	0.04	0.04	0.05
Vitamin K (µg)	0 (p=1)	(p=0.71)	(p=0.64)			-0.17	-0.17	-0.01	(p=0.01)		(p=0.24)	(p=0.99)	(p=0.65)	(p=0.67)	(p=0.57)
		-0.05	-0.07	(p=0.65)	(p=0.45)	(p=0.055)	(p=0.065)	(p=0.92)	0.11	(p=0.16)	0.07	(p=0.94)	(p=0.97)	(p=0.0043)	(p=0.0028)
LARN Vitamin K (%)				0.03	-0.08	-0.18	-0.17	-0.01							
		-0.06	-0.07	(p=0.75)	(p=0.39)	(p=0.053)	(p=0.056)	(p=0.89)	0.11	(p=0.18)	(p=0.42)	(p=0.89)	(p=0.97)	(p=0.0053)	(p=0.0031)
Vitamin E (mg)			-0.16	-0.05	-0.14	-0.21	-0.24	0.02	0.29	-0.54		-0.05			
		-0.16	(p=0.074)	(p=0.54)	(p=0.11)	(p=0.017)	(p=0.0071)	(p=0.83)	(p=0.00093)	(p=2.2e-11)	0.09	(p=0.54)	-0.01	-0.21	0.07
LARN Vitamin E (%)			-0.18	-0.1	-0.14	-0.22	-0.26	0	0.29	-0.53	0.09	-0.04	0	-0.23	
	-0.16	0	(p=0.036)	(p=0.26)	(p=0.11)	(p=0.014)	(p=0.0033)	(p=0.98)	(p=0.00093)	(p=1.1e-10)	(p=0.33)	(p=0.62)	(p=0.96)	(p=0.0087)	0.08
Thiamin (Vitamin B1) (mg)				0.08	-0.08	-0.16	-0.12	0.16							
		-0.07	0	(p=0.34)	(p=0.34)	(p=0.073)	(p=0.18)	(p=0.087)	0.14	(p=1.1e-10)	(p=0.22)	(p=0.055)	(p=0.85)	-0.05	0.11
LARN Thiamin (Vitamin B1) (%)				0.08	-0.08	-0.16	-0.12	0.16							
		-0.07	0	(p=0.34)	(p=0.34)	(p=0.073)	(p=0.18)	(p=0.087)	0.14	(p=1.1e-10)	(p=0.22)	(p=0.055)	(p=0.85)	-0.05	0.11
Riboflavin (Vitamin B2) (mg)				0.06	-0.01	-0.05	-0.02	0.13	0.15	-0.41	0.09	-0.2	0.08		
		-0.02	0.05	(p=0.49)	(p=0.87)	(p=0.61)	(p=0.83)	(p=0.16)	(p=0.086)	(p=1.2e-06)	(p=0.28)	(p=0.021)	(p=0.35)	-0.03	0.06
LARN Riboflavin (Vitamin B2) (%)				0.06	-0.01	-0.05	-0.02	0.13	0.15	-0.41	0.09	-0.2	0.08		
		-0.02	0.05	(p=0.49)	(p=0.87)	(p=0.61)	(p=0.83)	(p=0.16)	(p=0.086)	(p=1.2e-06)	(p=0.28)	(p=0.021)	(p=0.35)	-0.03	0.06
Niacin (Vitamin B3) (mg)				0.11	-0.05	-0.08	-0.04	0.09							
		-0.16	-0.01	(p=0.23)	(p=0.58)	(p=0.45)	(p=0.65)	(p=0.32)	0.05	(p=1.1e-05)	(p=0.46)	(p=0.014)	(p=0.29)	-0.07	0.08
LARN Niacin (Vitamin B3) (%)				-0.13	0.11	0.07	0	0.04							
		-0.08	0.02	(p=0.13)	(p=0.22)	(p=0.43)	(p=0.98)	(p=0.63)	-0.11	(p=0.28)	(p=0.47)	0.14	(p=0.0036)	(p=3.1e-05)	-0.14
Pantothenic acid (Vitamin B5) (mg)				0.12	-0.12	-0.14	-0.11	0.07							
		-0.13	-0.07	(p=0.18)	(p=0.16)	(p=0.11)	(p=0.21)	(p=0.47)	0.13	(p=1.2e-05)	(p=0.84)	(p=0.026)	0.01	0.07	0.09
LARN Pantothenic acid (Vitamin B5) (%)				0.12	-0.12	-0.14	-0.11	0.07							
		-0.13	-0.07	(p=0.18)	(p=0.16)	(p=0.11)	(p=0.21)	(p=0.47)	0.13	(p=1.2e-05)	(p=0.84)	(p=0.026)	0.01	0.07	0.09

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
Pyridoxine (Vitamin B6) (mg)	-0.11 (p=0.23)	-0.13 (p=0.13)	-0.04 (p=0.69)	0.11 (p=0.23)	-0.09 (p=0.3)	-0.09 (p=0.3)	-0.06 (p=0.5)	0.09 (p=0.34)	0.08 (p=0.37)	-0.57 (p=7.8e-13)	0.04 (p=0.6)	-0.24 (p=0.0059)	-0.02 (p=0.83)	-0.03 (p=0.73)	0.1 (p=0.25)
LARN Pyridoxine (Vitamin B6) (%)	-0.12 (p=0.16)	-0.14 (p=0.12)	-0.07 (p=0.42)	0.04 (p=0.63)	-0.1 (p=0.27)	-0.11 (p=0.22)	-0.1 (p=0.28)	0.07 (p=0.45)	0.09 (p=0.31)	-0.57 (p=9.7e-13)	0.03 (p=0.74)	-0.23 (p=0.0082)	-0.01 (p=0.94)	-0.06 (p=0.5)	0.11 (p=0.23)
Folic acid (µg)	-0.02 (p=0.83)	-0.1 (p=0.26)	0.03 (p=0.76)	-0.02 (p=0.81)	0.04 (p=0.67)	0.01 (p=0.93)	-0.01 (p=0.92)	0.07 (p=0.48)	0.33 (p=0.00012)	-0.69 (p=1.1e-19)	0.07 (p=0.44)	-0.09 (p=0.28)	0.05 (p=0.58)	-0.04 (p=0.65)	0.01 (p=0.93)
LARN Folic acid (%)	-0.02 (p=0.83)	-0.1 (p=0.26)	0.03 (p=0.76)	-0.02 (p=0.81)	0.04 (p=0.67)	0.01 (p=0.93)	-0.01 (p=0.92)	0.07 (p=0.48)	0.33 (p=0.00012)	-0.69 (p=1.1e-19)	0.07 (p=0.44)	-0.09 (p=0.28)	0.05 (p=0.58)	-0.04 (p=0.65)	0.01 (p=0.93)
Vitamin B12 (µg)	-0.04 (p=0.69)	0.03 (p=0.75)	0.12 (p=0.18)	0.16 (p=0.07)	0.03 (p=0.7)	0.07 (p=0.46)	0.12 (p=0.18)	0.22 (p=0.017)	-0.05 (p=0.56)	(p=0.28)	0.04 (p=0.6)	-0.05 (p=0.57)	-0.06 (p=0.52)	-0.03 (p=0.74)	-0.07 (p=0.44)
LARN Vitamin B12 (%)	-0.04 (p=0.69)	0.03 (p=0.75)	0.12 (p=0.18)	0.16 (p=0.07)	0.03 (p=0.7)	0.07 (p=0.46)	0.12 (p=0.18)	0.22 (p=0.017)	-0.05 (p=0.56)	(p=0.28)	0.04 (p=0.6)	-0.05 (p=0.57)	-0.06 (p=0.52)	-0.03 (p=0.74)	-0.07 (p=0.44)
Vitamin C (mg)	0.07 (p=0.45)	-0.14 (p=0.1)	0.06 (p=0.5)	-0.13 (p=0.14)	0.14 (p=0.11)	0.1 (p=0.27)	0.04 (p=0.67)	-0.03 (p=0.78)	0.33 (p=0.00013)	-0.64 (p=1.7e-16)	0.14 (p=0.11)	-0.07 (p=0.45)	0.06 (p=0.47)	0.03 (p=0.75)	0.04 (p=0.66)
LARN Vitamin C (%)	0.05 (p=0.57)	-0.14 (p=0.12)	0.01 (p=0.88)	-0.21 (p=0.017)	0.13 (p=0.13)	0.09 (p=0.33)		-0.06 (p=0.53)	0.34 (p=9e-05)	-0.62 (p=1.6e-15)	0.12 (p=0.17)	-0.04 (p=0.69)	0.08 (p=0.34)	-0.01 (p=0.94)	0.05 (p=0.6)
Minerals															
Sodium (g)	-0.04 (p=0.65)	-0.1 (p=0.28)	0.08 (p=0.37)	0.21 (p=0.017)	-0.02 (p=0.84)	-0.05 (p=0.57)	0.03 (p=0.77)	0.1 (p=0.29)	-0.07 (p=0.43)	0.07 (p=0.4)	0.18 (p=0.043)	-0.26 (p=0.0023)	0.01 (p=0.94)	-0.11 (p=0.2)	0.1 (p=0.28)
LARN Sodium (%)	-0.04 (p=0.65)	-0.1 (p=0.28)	0.08 (p=0.37)	0.21 (p=0.017)	-0.02 (p=0.84)	-0.05 (p=0.57)	0.03 (p=0.77)	0.1 (p=0.29)	-0.07 (p=0.43)	0.07 (p=0.4)	0.18 (p=0.043)	-0.26 (p=0.0023)	0.01 (p=0.94)	-0.11 (p=0.2)	0.1 (p=0.28)
Potassium (g)	-0.09 (p=0.28)	-0.18 (p=0.041)	-0.08 (p=0.39)	0.07 (p=0.45)	-0.13 (p=0.15)	-0.15 (p=0.099)	-0.13 (p=0.15)	0.04 (p=0.63)	0.24 (p=0.0062)	-0.72 (p=4e-22)	0.11 (p=0.2)	0.01 (p=0.014)	0.07 (p=0.42)	-0.04 (p=0.63)	0.17 (p=0.065)
LARN Potassium (%)	-0.09 (p=0.28)	-0.18 (p=0.041)	-0.08 (p=0.39)	0.07 (p=0.45)	-0.13 (p=0.15)	-0.15 (p=0.099)	-0.13 (p=0.15)	0.04 (p=0.63)	0.24 (p=0.0062)	-0.72 (p=4e-22)	0.11 (p=0.2)	0.01 (p=0.014)	0.07 (p=0.42)	-0.04 (p=0.63)	0.17 (p=0.065)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
Calcium (mg)	0.1 (p=0.28)	-0.13 (p=0.15)	0.1 (p=0.24)	0.23 (p=0.081)	-0.04 (p=0.65)	0 (p=0.97)	0.08 (p=0.38)	0.08 (p=0.38)	0.06 (p=0.49)	-0.16 (p=0.071)	0.09 (p=0.33)	-0.18 (p=0.045)	0.21 (p=0.015)	0.16 (p=0.06)	0.1 (p=0.26)
LARN Calcium (%)	0.1 (p=0.28)	-0.13 (p=0.15)	0.1 (p=0.24)	(p=0.081)	-0.04 (p=0.65)	0 (p=0.97)	0.08 (p=0.38)	0.08 (p=0.38)	0.06 (p=0.49)	-0.16 (p=0.071)	0.09 (p=0.33)	-0.18 (p=0.045)	0.21 (p=0.015)	0.16 (p=0.06)	0.1 (p=0.26)
Magnesium (mg)	-0.08 (p=0.34)	-0.15 (p=0.093)	0.16 (p=0.84)	-0.11 (p=0.061)	-0.15 (p=0.19)	-0.09 (p=0.32)	0.09 (p=0.32)	0.15 (p=0.085)	-0.44 (p=1.5e-07)	-0.04 (p=0.66)	-0.15 (p=0.092)	-0.01 (p=0.89)	0.01 (p=0.87)	0.03 (p=0.87)	0.03 (p=0.72)
LARN Magnesium (%)	-0.08 (p=0.34)	-0.15 (p=0.093)	0.16 (p=0.84)	-0.11 (p=0.061)	-0.15 (p=0.19)	-0.09 (p=0.32)	0.09 (p=0.32)	0.15 (p=0.085)	-0.44 (p=1.5e-07)	-0.04 (p=0.66)	-0.15 (p=0.092)	-0.01 (p=0.89)	0.01 (p=0.87)	0.03 (p=0.87)	0.03 (p=0.72)
Phosphorus (mg)	-0.1 (p=0.24)	-0.14 (p=0.12)	0.02 (p=0.85)	0.27 (p=0.02)	-0.13 (p=0.14)	-0.09 (p=0.33)	0 (p=0.96)	0.2 (p=0.028)	0.05 (p=0.53)	-0.33 (p=0.0013)	0.01 (p=0.9)	-0.27 (p=0.0019)	0.08 (p=0.34)	0.09 (p=0.29)	0.08 (p=0.4)
LARN Phosphorus (%)	-0.1 (p=0.24)	-0.14 (p=0.12)	0.02 (p=0.85)	(p=0.002)	-0.13 (p=0.14)	-0.09 (p=0.33)	0 (p=0.96)	0.2 (p=0.028)	0.05 (p=0.53)	-0.33 (p=0.0013)	0.01 (p=0.9)	-0.27 (p=0.0019)	0.08 (p=0.34)	0.09 (p=0.29)	0.08 (p=0.4)
Iron (mg)	-0.06 (p=0.49)	-0.12 (p=0.19)	0.02 (p=0.83)	0.1 (p=0.24)	-0.04 (p=0.62)	-0.07 (p=0.43)	0.08 (p=0.737)	0.08 (p=0.37)	0.13 (p=0.14)	(p=1.9e-14)	0.08 (p=0.34)	-0.12 (p=0.18)	0.05 (p=0.57)	0.05 (p=0.61)	0.02 (p=0.79)
LARN Iron (%)	-0.06 (p=0.49)	-0.12 (p=0.19)	0.02 (p=0.83)	0.1 (p=0.24)	-0.04 (p=0.62)	-0.07 (p=0.43)	0.08 (p=0.737)	0.08 (p=0.37)	0.13 (p=0.14)	(p=1.9e-14)	0.08 (p=0.34)	-0.12 (p=0.18)	0.05 (p=0.57)	0.05 (p=0.61)	0.02 (p=0.79)
Zinc (mg)	-0.1 (p=0.24)	-0.13 (p=0.13)	0.09 (p=0.3)	0.3 (p=0.0048)	-0.05 (p=0.56)	-0.07 (p=0.43)	0.04 (p=0.64)	0.25 (p=0.006)	-0.01 (p=0.87)	(p=0.0014)	0.1 (p=0.25)	-0.25 (p=0.0036)	0.07 (p=0.42)	0.13 (p=0.14)	0 (p=0.99)
LARN Zinc (%)	-0.15 (p=0.089)	-0.11 (p=0.2)	-0.01 (p=0.88)	0.11 (p=0.1)	-0.06 (p=0.47)	-0.1 (p=0.27)	-0.05 (p=0.56)	0.2 (p=0.029)	0.02 (p=0.82)	(p=0.0047)	0.07 (p=0.43)	-0.21 (p=0.004)	0.11 (p=0.23)	0.05 (p=0.54)	0.04 (p=0.67)
Copper (mg)	0.03 (p=0.76)	(p=0.032)	0.03 (p=0.71)	(p=0.0054)	-0.09 (p=0.33)	-0.12 (p=0.2)	-0.04 (p=0.69)	0.1 (p=0.3)	0.07 (p=0.4)	-0.28 (p=0.0011)	0.05 (p=0.54)	-0.09 (p=0.29)	0.09 (p=0.28)	0.27 (p=0.0015)	0.01 (p=0.93)
LARN Copper (%)	0.02 (p=0.86)	-0.18 (p=0.036)	0.01 (p=0.92)	0.2 (p=0.023)	-0.09 (p=0.31)	-0.12 (p=0.18)	-0.06 (p=0.54)	0.09 (p=0.35)	0.09 (p=0.33)	(p=9e-04)	0.05 (p=0.6)	-0.08 (p=0.38)	0.1 (p=0.25)	0.25 (p=0.004)	0.02 (p=0.79)
Manganese (mg)	-0.08 (p=0.34)	-0.02 (p=0.86)	-0.03 (p=0.71)	0.16 (p=0.074)	-0.1 (p=0.24)	-0.15 (p=0.1)	-0.1 (p=0.27)	-0.03 (p=0.72)	0.23 (p=0.0081)	-0.34 (p=7.4e-05)	0 (p=1)	-0.06 (p=0.51)	-0.06 (p=0.48)	0.08 (p=0.38)	-0.02 (p=0.86)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
LARN Manganese (%)	-0.1 (p=0.27)	-0.02 (p=0.84)	-0.05 (p=0.54)	0.13 (p=0.14)	-0.11 (p=0.2)	-0.16 (p=0.083)	-0.12 (p=0.19)	-0.04 (p=0.7)	0.24 (p=0.0059)	-0.34 (p=7.6e-05)	-0.01 (p=0.95)	-0.05 (p=0.58)	-0.05 (p=0.57)	0.07 (p=0.43)	-0.01 (p=0.87)
Iodine (µg)	-0.07 (p=0.45)	-0.07 (p=0.41)	-0.03 (p=0.77)	0.15 (p=0.089)	-0.11 (p=0.2)	-0.09 (p=0.34)	-0.04 (p=0.68)	0.07 (p=0.44)	0.06 (p=0.47)	(p=0.31)	(p=0.32)	-0.06 (p=0.5)	(p=0.12)	-0.09 (p=0.33)	0.13 (p=0.15)
LARN Iodine (%)	-0.07 (p=0.45)	-0.07 (p=0.41)	-0.03 (p=0.77)	0.15 (p=0.089)	-0.11 (p=0.2)	-0.09 (p=0.34)	-0.04 (p=0.68)	0.07 (p=0.44)	0.06 (p=0.47)	(p=0.31)	(p=0.32)	-0.06 (p=0.5)	(p=0.12)	-0.09 (p=0.33)	0.13 (p=0.15)
Selenium (µg)	-0.12 (p=0.16)	0 (p=0.96)	0.02 (p=0.83)	0.18 (p=0.04)	-0.1 (p=0.25)	-0.11 (p=0.23)	-0.04 (p=0.67)	0.17 (p=0.062)	0.16 (p=0.065)	-0.2 (p=0.02)	0.02 (p=0.81)	-0.13 (p=0.14)	0 (p=0.99)	-0.04 (p=0.68)	0.08 (p=0.36)
Carbohydrates															
Glucose (g)	0.28 (p=0.0014)	-0.08 (p=0.38)	0 (p=0.97)	0.11 (p=0.21)	-0.06 (p=0.52)	-0.03 (p=0.78)	-0.01 (p=0.9)	-0.11 (p=0.21)	0.19 (p=0.026)	-0.41 (p=1e-06)	0.11 (p=0.2)	-0.01 (p=0.89)	0.06 (p=0.53)	0.14 (p=0.11)	0.1 (p=0.27)
Fructose (g)	0.23 (p=0.0088)	-0.08 (p=0.37)	-0.01 (p=0.88)	0.09 (p=0.3)	-0.03 (p=0.7)	0 (p=0.9)	-0.01 (p=0.9)	-0.15 (p=0.11)	0.15 (p=0.096)	-0.31 (p=0.00026)	0.15 (p=0.095)	-0.09 (p=0.33)	0.07 (p=0.44)	0.14 (p=0.1)	0.12 (p=0.17)
Sucrose (g)	0.17 (p=0.051)	-0.04 (p=0.68)	0.02 (p=0.84)	0.11 (p=0.23)	-0.04 (p=0.6)	0.06 (p=0.52)	0.08 (p=0.36)	-0.05 (p=0.6)	-0.19 (p=0.029)	-0.13 (p=0.14)	0 (p=0.97)	0.14 (p=0.12)	-0.19 (p=0.028)	0.03 (p=0.69)	-0.22 (p=0.013)
Lactose (g)	-0.05 (p=0.56)	-0.21 (p=0.015)	-0.11 (p=0.2)	0 (p=0.99)	-0.16 (p=0.066)	-0.1 (p=0.26)	-0.07 (p=0.42)	0.01 (p=0.91)	-0.2 (p=0.024)	0.04 (p=0.66)	0.01 (p=0.91)	-0.12 (p=0.18)	0.12 (p=0.17)	0.02 (p=0.82)	0.02 (p=0.82)
Total sugars (g)	0.05 (p=0.57)	-0.05 (p=0.6)	-0.01 (p=0.93)	0.21 (p=0.014)	-0.13 (p=0.15)	-0.01 (p=0.91)	0.06 (p=0.5)	-0.11 (p=0.25)	-0.35 (p=4.4e-05)	0.16 (p=0.063)	-0.04 (p=0.68)	0.08 (p=0.37)	-0.03 (p=0.7)	0.15 (p=0.081)	-0.11 (p=0.22)
Fats															
SFA (g)	0 (p=0.98)	-0.13 (p=0.15)	0.02 (p=0.8)	0.22 (p=0.01)	-0.08 (p=0.34)	-0.06 (p=0.52)	0.02 (p=0.84)	0.07 (p=0.46)	-0.2 (p=0.023)	0.13 (p=0.13)	0.17 (p=0.059)	-0.06 (p=0.48)	0.02 (p=0.85)	0.11 (p=0.19)	0.02 (p=0.82)
MUFA (g)	-0.13 (p=0.14)	-0.07 (p=0.41)	-0.05 (p=0.57)	0.05 (p=0.5)	-0.09 (p=0.29)	-0.17 (p=0.057)	-0.15 (p=0.093)	0.02 (p=0.87)	0.1 (p=0.24)	-0.23 (p=0.0085)	0.04 (p=0.63)	-0.07 (p=0.41)	0.01 (p=0.87)	-0.05 (p=0.54)	0.04 (p=0.67)
Linolic FA (g)	-0.1 (p=0.26)	0.06 (p=0.5)	-0.15 (p=0.096)	0.15 (p=0.09)	-0.25 (p=0.039)	-0.24 (p=0.087)	-0.17 (p=0.06)	0.07 (p=0.47)	0.1 (p=0.27)	-0.26 (p=0.0031)	-0.03 (p=0.77)	0.01 (p=0.94)	-0.15 (p=0.088)	-0.18 (p=0.046)	0.01 (p=0.89)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
Linolenic FA (g)	-0.06 (p=0.5)	0.02 (p=0.85)	-0.07 (p=0.41)	0.1 (p=0.25)	-0.13 (p=0.14)	-0.13 (p=0.14)	-0.11 (p=0.23)	0.06 (p=0.48)	0.2 (p=0.022)	-0.35 (p=0.36e-05)	0.09 (p=0.29)	-0.09 (p=0.28)	-0.1 (p=0.25)	-0.03 (p=0.71)	0.08 (p=0.36)
PUFA (g)	-0.05 (p=0.55)	0.09 (p=0.32)	-0.12 (p=0.17)	0.15 (p=0.082)	-0.22 (p=0.013)	-0.21 (p=0.022)	-0.15 (p=0.11)	0.07 (p=0.44)	0.13 (p=0.14)	(p=0.0023)	-0.03 (p=0.77)	0 (p=0.97)	-0.15 (p=0.092)	-0.14 (p=0.12)	0.05 (p=0.57)
Cholesterol (g)	-0.14 (p=0.12)	-0.12 (p=0.16)	0.08 (p=0.34)	0.13 (p=0.13)	0.03 (p=0.7)	(p=0.86)	(p=0.52)	(p=0.1124)	-0.13 (p=0.14)	0.05 (p=0.6)	(p=0.86)	(p=0.35)	(p=0.43)	0.04 (p=0.63)	-0.04 (p=0.64)
Flavonoids															
Flavan-3 ols (mg)	-0.11 (p=0.21)	-0.22 (p=0.012)	0.04 (p=0.68)	0.12 (p=0.16)	-0.05 (p=0.54)	-0.06 (p=0.51)	0 (p=0.99)	0.04 (p=0.65)	-0.13 (p=0.14)	(p=0.0013)	-0.03 (p=0.72)	-0.01 (p=0.93)	-0.02 (p=0.84)	0.09 (p=0.29)	0.08 (p=0.38)
Flavones (mg)	0.04 (p=0.62)	-0.05 (p=0.57)	0.05 (p=0.58)	-0.06 (p=0.46)	0.08 (p=0.37)	-0.01 (p=0.89)	-0.04 (p=0.7)	-0.12 (p=0.21)	0.19 (p=0.029)	-0.49 (p=0.34e-09)	0.11 (p=0.23)	-0.08 (p=0.35)	-0.04 (p=0.67)	-0.04 (p=0.65)	-0.1 (p=0.28)
Flavonols (mg)	-0.06 (p=0.51)	-0.14 (p=0.12)	0.09 (p=0.33)	0.06 (p=0.5)	0.06 (p=0.4)	0.05 (p=0.57)	0.08 (p=0.38)	0.06 (p=0.51)	0.06 (p=0.52)	(p=2.9e-08)	(p=0.69)	(p=0.26)	(p=0.83)	0.12 (p=0.18)	-0.11 (p=0.21)
Flavonones (mg)	-0.14 (p=0.12)	-0.14 (p=0.11)	-0.02 (p=0.79)	0.07 (p=0.43)	-0.04 (p=0.68)	-0.07 (p=0.47)	-0.06 (p=0.53)	0.19 (p=0.038)	-0.03 (p=0.75)	(p=0.11)	(p=0.14)	(p=0.41)	(p=0.66)	0.08 (p=0.35)	0.03 (p=0.76)
Anthocyanidins (mg)	0.03 (p=0.73)	-0.1 (p=0.28)	0.1 (p=0.24)	0.05 (p=0.61)	0.08 (p=0.34)	0.05 (p=0.59)	0.08 (p=0.4)	0.19 (p=0.035)	0.04 (p=0.69)	(p=0.051)	(p=0.39)	(p=0.038)	(p=0.14)	0.13 (p=0.13)	-0.06 (p=0.52)
Isoflavones (mg)	-0.03 (p=0.76)	-0.06 (p=0.51)	0.23 (p=0.0078)	0.22 (p=0.013)	0.12 (p=0.16)	0.1 (p=0.27)	0.16 (p=0.072)	0.17 (p=0.065)	-0.12 (p=0.16)	0.02 (p=0.18)	-0.09 (p=0.84)	-0.09 (p=0.33)	0.04 (p=0.64)	0.14 (p=0.11)	-0.14 (p=0.13)
		0.32 (p=0.00023)	0.15 (p=0.083)	-0.05 (p=0.56)	0.19 (p=0.013)	0.38 (p=0.0016e-05)	0.35 (p=0.08e-05)	-0.3 (p=0.0011)	0 (p=0.99)	0.04 (p=0.68)	-0.06 (p=0.53)	0.11 (p=0.23)	-0.04 (p=0.69)	0.08 (p=0.39)	0.12 (p=0.2)
Age (y)	1 (p=0)	0.32 (p=0.00023)	0.01 (p=0.95)	-0.05 (p=0.56)	0.09 (p=0.3)	0.21 (p=0.018)	0.15 (p=0.093)	-0.29 (p=0.0014)	0 (p=0.96)	0.12 (p=0.17)	-0.22 (p=0.011)	0.24 (p=0.0066)	-0.34 (p=0.006e-05)	-0.31 (p=0.00031)	0.05 (p=0.59)
EDSS	1 (p=0)	1 (p=0)	0.01 (p=0.95)	0.01 (p=0.95)	0.03 (p=0.2)	(p=0.018)	(p=0.093)	(p=0.0014)	(p=0.96)	(p=0.17)	(p=0.011)	(p=0.0066)	(p=0.006e-05)	(p=0.00031)	0.05 (p=0.59)
Weight (kg)	0.15 (p=0.083)	0.01 (p=0.95)	1 (p=0)	(p=1.1e-06)	(p=4.5e-40)	(p=1.1e-25)	(p=1.4e-42)	(p=0.061)	-0.03 (p=0.71)	(p=0.94)	(p=0.94)	(p=0.12)	(p=0.72)	0.15 (p=0.093)	-0.21 (p=0.02)

	Age (y)	EDSS	Weight (kg)	Height (m)	BMI (kg/m ²)	WhtR	WC (cm)	Phase angle °	Medi-Lite	DII	IPAQ	PSQI	MSQoL-29 physical	MSQoL-29 mental	25(OH)D (ng/ml)
			0.41		-0.04	-0.07	0.24	0.23		-0.01	0.08	-0.27		0.24	
Height (m)	-0.05 (p=0.56)	-0.05 (p=0.56)	(p=1.1e-06)	1 (p=0)	(p=0.64)	(p=0.44)	(p=0.07)	(p=0.013)	(p=0.91)	(p=0.91)	(p=0.37)	(p=0.0019)	-0.02 (p=0.8)	(p=0.0053)	-0.07 (p=0.43)
			0.86	-0.04		0.89	0.84	0.06		0.02	-0.05	-0.01	0.02		-0.18
BMI (kg/m ²)	0.19 (p=0.033)	0.09 (p=0.32)	(p=4.5e-40)	(p=0.64)	1 (p=0)	(p=2e-43)	(p=9.4e-34)	(p=0.55)	(p=0.61)	(p=0.82)	(p=0.55)	(p=0.92)	(p=0.83)	0.03 (p=0.7)	(p=0.047)
	0.38 (p=1.6e-05)	0.21 (p=0.018)	0.77 (p=1.1e-25)	-0.07 (p=0.44)	0.89 (p=2e-43)		0.94 (p=3.7e-59)	-0.04 (p=0.64)		0.11	-0.15		-0.06		
WhtR			0.89	0.24	0.84	0.94		0.04	(p=0.27)	(p=0.23)	(p=0.091)	0.09 (p=0.3)	(p=0.53)	-0.01 (p=0.92)	-0.15 (p=0.11)
	0.35 (p=8e-05)	0.15 (p=0.093)	(p=1.4e-42)	(p=0.07)	(p=9.4e-34)	(p=3.7e-59)		(p=0.67)	(p=0.26)	(p=0.24)	(p=0.16)	(p=0.94)	(p=0.55)	0.07 (p=0.45)	(p=0.072)
WC (cm)	-0.3 (p=0.0011)	-0.29 (p=0.0014)	0.17 (p=0.061)	0.23 (p=0.013)	0.06 (p=0.55)	-0.04 (p=0.64)	0.04 (p=0.67)			-0.04	0.17	-0.2 (p=0.029)	0.13 (p=0.16)	0.11 (p=0.23)	-0.22 (p=0.019)
Phase angle °				-0.01	-0.05	-0.1	-0.1	0	(p=0.96)	(p=0.66)	(p=0.066)	(p=0.029)	(p=0.16)	0.11 (p=0.23)	
		0	-0.03	(p=0.91)	(p=0.61)	(p=0.27)	(p=0.26)	(p=0.96)		(p=4.6e-06)	(p=0.36)	(p=0.31)	(p=0.017)	0.06 (p=0.48)	0.1 (p=0.27)
Medi-Lite	0 (p=0.99)	(p=0.96)	(p=0.71)	-0.01	0.02	0.11	0.11	-0.04	(p=4.6e-06)		-0.11	0.16	-0.02		
	0.04 (p=0.68)	0.12 (p=0.17)	-0.01 (p=0.94)	(p=0.91)	(p=0.82)	(p=0.23)	(p=0.24)	(p=0.66)		(p=0.1)	(p=0.069)	(p=0.065)	(p=0.78)	-0.02 (p=0.81)	-0.04 (p=0.7)
DII		-0.22		0.08	-0.05	-0.15	-0.13	0.17		-0.11		-0.25	0.17		
	-0.06 (p=0.53)	(p=0.011)	-0.01 (p=0.94)	(p=0.37)	(p=0.55)	(p=0.091)	(p=0.16)	(p=0.066)	(p=0.36)	(p=0.19)	1 (p=0)	(p=0.0043)	(p=0.05)	0.23 (p=0.009)	0.04 (p=0.67)
IPAQ		0.24	-0.27	-0.01	-0.01	-0.2		-0.2		0.16	-0.25		-0.13	-0.39	
	0.11 (p=0.23)	(p=0.0066)	-0.14 (p=0.12)	(p=0.019)	(p=0.92)	0.09 (p=0.3)	(p=0.94)	(p=0.029)	(p=0.31)	(p=0.065)	(p=0.0043)	1 (p=0)	(p=0.13)	(p=5.2e-06)	-0.03 (p=0.78)
PSQI		-0.34	-0.02	0.02	-0.06	-0.05	-0.05	0.13		-0.02	0.17	-0.13		0.49	
	-0.04 (p=0.69)	(p=6.4e-05)	0.03 (p=0.72)	(p=0.8)	(p=0.83)	(p=0.53)	(p=0.55)	(p=0.16)	(p=0.017)	(p=0.78)	(p=0.05)	(p=0.13)	1 (p=0)	(p=1.9e-09)	0.08 (p=0.39)
MSQoL-29 physical		-0.31	0.15	0.24		-0.01	0.07	0.11		-0.02	0.23	-0.39	0.49		
	0.08 (p=0.39)	(p=0.0031)	(p=0.093)	(p=0.053)	0.03 (p=0.7)	(p=0.92)	(p=0.45)	(p=0.23)	(p=0.48)	(p=0.81)	(p=0.009)	(p=5.2e-06)	(p=1.9e-09)	1 (p=0)	-0.01 (p=0.91)
MSQoL-29 mental			-0.07	-0.18	-0.15	-0.17	-0.22				0.04	-0.03	0.08		
	0.12 (p=0.2)	0.05 (p=0.59)	-0.21 (p=0.02)	(p=0.43)	(p=0.047)	(p=0.11)	(p=0.072)	(p=0.019)	(p=0.27)	-0.04 (p=0.7)	(p=0.67)	(p=0.78)	(p=0.39)	-0.01 (p=0.91)	1 (p=0)
25(OH)D (ng/ml)															

*statistically significant P-value<0.05

BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; NEDA-3: No Evidence of Disease Activity; WhtR: Waist-to-Height Ratio; WC: Waist Circumference; MSQoL-29: Multiple Sclerosis Quality of life 29 questionnaire; DII: Dietary Inflammatory Index; IPAQ: International Physical Activity Questionnaire; METs: Metabolic Equivalents; PA: Physical Activity; PSQI: Pittsburgh Sleep Quality Index; 25(OH)D: 25-hydroxy vitamin D;

Phase angle associations with predictors identified by LASSO regression

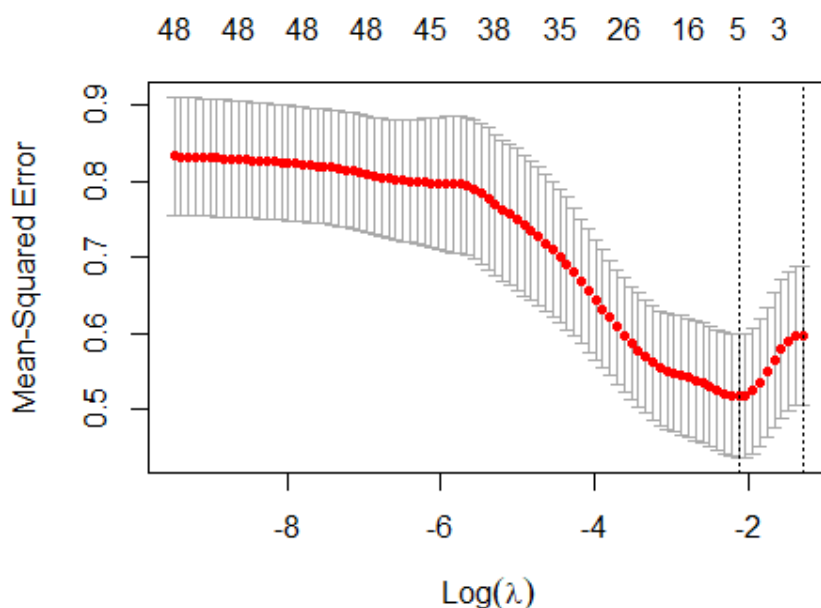


Figure 4 Cross-validation curve for Lasso regression model predicting phase angle. The plot displays the mean squared error (MSE) for different values of the regularization parameter $\log(\lambda)$. Each red dot represents the average MSE across cross-validation folds, and the gray error bars show ± 1 standard error.

The model returned an Adjusted $R^2 = 0.31$: the model explains about 31% of the observed variability, suggesting a good predictive capacity. Although not high, this value is consistent with the analysis of complex and multifactorial problems such as biological and nutritional ones. The LASSO regression identified a number of predictive variables ranging from 6 to 0, depending on the chosen penalization criterion (Figure 3). To balance parsimony and predictive capacity, 5 variables were selected to be included in the multiple linear regression on phase angle. From the analysis, it was found that dietary selenium (Se) intake was positively associated to higher phase angle, specifically an increase of 1 μg of selenium intake may result in an increase of 0.006° of phase angle (P-value = 0.030), holding all other factors in the model constant. Age was negatively associated to phase angle, meaning that on average, for each additional year of age, the phase angle decreases by about 0.0155° (P-value=0.004). Physical activity (IPAQ) was also associated with phase angle, but with little clinical relevance, although statistically significant. Finally, being female showed a negative relationship with phase angle (P-value = 0.007) (Table 8).

Table 9 Associations of phase angle predictors identified by LASSO regression

	β (95% CI)	P-value*
Age	-0.01549 (-0.02578, -0.00520)	0.004
Female sex	-0.36308 (-0.62479, -0.10138)	0.007
EDSS	-0.07710 (-0.16234, 0.00813)	0.076
IPAQ	0.00007 (0.00003, 0.00010)	<0.001
Selenium	0.00523 (0.00040, 0.01006)	0.034

*statistically significant P-value<0.05; Variables with non-zero coefficients after penalty tuning are shown.

DII associations with predictors identified by LASSO regression

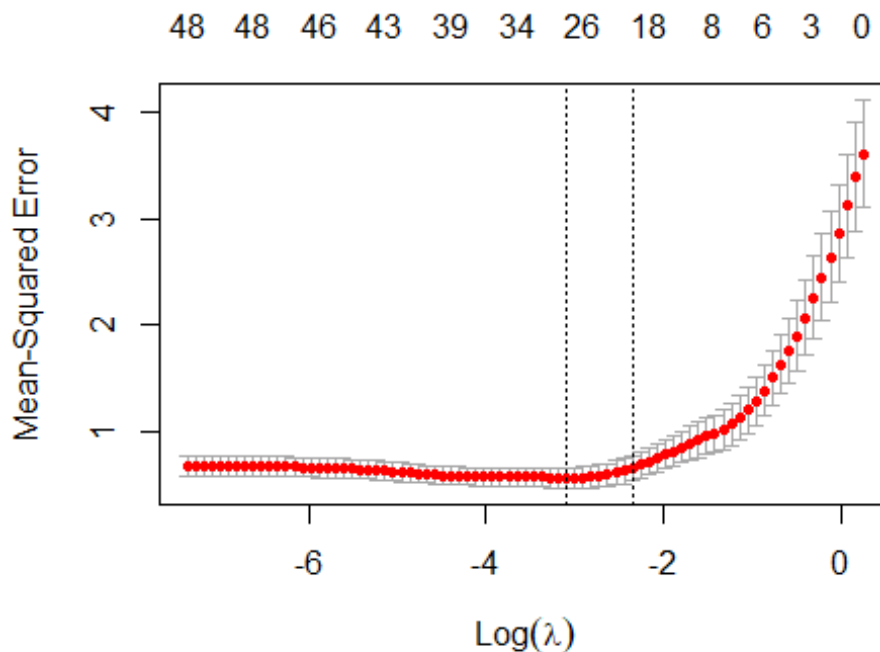


Figure 5 Cross-validation curve for Lasso regression model predicting DII. The plot displays the mean squared error (MSE) for different values of the regularization parameter $\log(\lambda)$. Each red dot represents the average MSE across cross-validation folds, and the gray error bars show ± 1 standard error.

The LASSO regression model predicting DII returned an Adjusted R^2 of 0.875, indicating excellent overall predictive ability. The minimum MSE was around $\text{Log}(\lambda) \approx -3.5$, which corresponds between 29 and 20 variables (Figure 4). The model showed multiple associations of nutrients and DII. Because several of these variables selected by LASSO are themselves components of the DII calculation, their strong associations with DII reflect the underlying formula and should not be interpreted as independent predictors. These results essentially confirm that DII behaves as expected with respect to its component nutrients. Of particular interest is the statistically significant negative association of 25(OH)D and DII. Specifically, for each 1 ng/ml increase of 25(OH)D, there is reduction in the DII

of 0.011 units, a statistically significant effect (P-value = 0.031). For example, if an individual with vitamin D deficiency (20 µg/ml) reaches optimal levels of 50 µg/ml, the model predicts a decrease in the DII of approximately –0.33 points. On the other hand, the socio-demographic variables suggested by the LASSO regression were not found to be associated with DII (Table 9).

Table 10 Associations of DII predictors identified by LASSO regression

	β (95% CI)	P-value
NEDA 3 (Yes)	-0.34535 (-0.87040, 0.17970)	0.1945
Education (Highschool)	-0.24499 (-0.60476, 0.11478)	0.180
Education (BSc degree)	-0.26879 (-0.67024, 0.13266)	0.187
Education (MSc degree)	-0.19299 (-1.03052, 0.64455)	0.648
25(OH)D	-0.01053 (-0.01836, -0.00270)	0.009
MSQoL 29 physical	-0.00332 (-0.01479, 0.00816)	0.567
Medi-Lite	-0.05412 (-0.12478, 0.01654)	0.132
Alcohol	-0.02950 (-0.05081, -0.00819)	0.007
Fiber	-0.07589 (-0.09828, -0.05349)	0.000
Vitamin E	-0.08597 (-0.12538, -0.04656)	0.000
Vitamin B12	0.00579 (-0.05821, 0.06979)	0.858
Vitamin A	-0.00017 (-0.00049, 0.00016)	0.309
Folic acid	-0.00379 (-0.00537, -0.00221)	0.000
Sodium	0.00007 (-0.00007, 0.00021)	0.306
Total sugars	0.00089 (-0.00396, 0.00574)	0.715
SFA	0.02940 (0.01597, 0.04284)	0.000
Flavan 3-ols	-0.00121 (-0.00185, -0.00056)	0.000
Flavones	-0.58380 (-0.70191, -0.46568)	0.000
Flavonols	-0.00832 (-0.01105, -0.00559)	0.000
Flavonones	-0.05367 (-0.09442, -0.01292)	0.010
Anthocyanidins	-0.00571 (-0.01286, 0.00144)	0.116
Isoflavones	-0.00934 (-0.01683, -0.00184)	0.015

**statistically significant P-value<0.05; Variables with non-zero coefficients after penalty tuning are shown.*

Chapter 5 Discussion

Overall nutritional and lifestyle assessment and sex-specific differences regarding nutritional intake and lifestyle in pwMS

This thesis analyzed the overall external exposome factors of pwMS focusing on dietary habits and showed that overall dietary patterns reflected a good adherence to the Mediterranean diet (median Medi-Lite score 11), though the population reported a highly pro-inflammatory diet (median DII 4.1) with low consumption in plant-based food, such as fruit, vegetable and legumes, and fish and high consumption of red meat and confectionary products. This population was deficient in multiple nutrient intakes of vitamins and minerals. Vitamin D insufficiency was common, with a median serum 25(OH)D of 22 ng/ml despite frequent supplement use, and sleep quality was generally poor, especially among women. Regression analyses showed that higher selenium intake and physical activity were associated with higher phase angle, whereas older age and female sex predicted lower values. Additionally, higher serum 25(OH)D was independently linked to lower DII scores, suggesting a role of vitamin D in modulating the inflammatory potential of the diet.

A noteworthy observation was that higher intake of healthy nutrients, such as protein, fiber, riboflavin, pyridoxine, pantothenic acid, calcium and others were correlated with better sleep quality in pwMS. In general, it is reported by a systematic review that a healthier diet and better diet quality can contribute to a better sleep quality (184). Moreover, higher PA was correlated with better sleep quality and better mental and physical QoL in pwMS, which has already been demonstrated in different populations (185,186). As it could be hypothesized, lower disability scores of EDSS were correlated with better QoL, both physical and mental, higher PA and better sleep quality, showing how disability can affect people's life in different lifestyle dimensions (187). Higher alcohol consumption was paradoxically correlated with lower EDSS. This has been shown by other studies, too. For example, a case-control study in Sweden showed that low and moderate alcohol drinking had better outcomes regarding the RRMS and disability mostly among women, compared to nondrinking, while there were no significant outcomes with high alcohol intake (108), but it should be considered that alcohol consumption is an exposure to multiple health-related risks and diseases and there is no therapeutic dosage for the immune system suppression (107). Another worth mentioning result was that higher sucrose intake and higher BMI were correlated to lower 25(OH)D. There are some studies supporting these results, but not in pwMS. A study conducted in premenopausal women in Canada showed that a higher intake of colas was associated with lower mean of 25(OH)D (188), while another study using data from NHANES in U.S. adults concluded that BMI mediates the inverse association between sugar-sweetened beverages and 25(OH)D (189).

As mentioned before, MS occurs differently in men and women regarding symptoms, age and prognosis of the disease (7) and sometimes lifestyle habits as a result of MS. In general, low mental health, depression, anxiety and negative emotions are more common in pwMS compared to the general population (148). Specifically in this analysis, it was found that women with MS have significantly lower mental-related QoL. A multicenter cross-sectional study in China with 931 pwMS comes partially in accordance with these results showing that nonacceptance, sadness and sense of helpless was more likely in women (190). On the other, hand a study in Canada with 743 pwMS for at least 20 years showed that depression was more common in men, while anxiety in women (191). No statistically significant differences were found regarding PA in pwMS when sexes were compared, in contrast to other studies that found that men were significantly more active than women (127–129). The study in Canada mentioned above though, found no differences in PA, too (191). Sleep disturbances and low sleep quality are prevalent in pwMS compared to the general population and other chronic diseases. This is in agreement with a study from USA including 1063 pwMS that women have statistically significantly lower sleep adequacy than men (133), while another study including 153 pwMS from Slovakia showed no gender specific differences about sleep quality, but showed that the reasons of poor sleep quality differ between men and women with MS (192). Additionally, the vitamin D status of pwMS shows insufficiency, even though more than half of the participants were supplemented, however at least in an observational Polish study showed that pwMS have better vitamin D status than the general population (193). This study showed that women with MS had significantly higher 25(OH)D than men. These results align with the only study found making this comparison by Barnes et al. (2007) conducted in Ireland, in which it was found that women with MS had significantly higher 25(OH)D and 1,25(OH)₂D₃ (the active form of vitamin D) concentrations than men (194). Regarding nutrient intake differences, no study has compared the vitamin E percentage of intake compared to recommendations. In the specific study, it was found that women met a significantly higher percentage of the vitamin E intake recommendations according to LARN. The national survey of Korea from 2016-2019 showed that women met significantly less frequently the national recommendations of adequate intake for vitamin E, however it should be considered that every country may have different nutrient intake guidelines (195). In multiple European countries lower vitamin E intake is recommended for women (196), while in countries such as USA equal vitamin E is suggested (197). Regarding vitamin B12 intake, it was shown that male had a median intake that was closer to LARN recommendations. No study analysing the sex-specific of vitamin B12 intake in pwMS is available. A study by Rippin et al (2017),

including surveys from 21 European countries including Italy, showed that the vitamin B12 intake was overall higher in men, however it was not specified if the differences were significant (198). According to the latest Italian nutrition survey, men consumed higher amounts of meat and animal products containing high concentrations of vitamin B12, hence the results of this study, too (199). No study has evaluated the sex-differences regarding phosphorus intake, but in the specific analysis, it was found that males with MS had significantly higher percentage in meeting phosphorus intake recommendations. The nutrition survey of USA (NHANES) showed that in the general population the phosphorus intake in men was significantly higher than in women (200). As mentioned before, Italian men tend to eat more meat and dairy than women and phosphorus is rich in these products, so this may explain the results of our study (201). With the same reasoning, the copper intake as recommendation percentage was higher in men than women with MS, since men tend to eat higher amounts of protein-rich foods, meat and seafood containing copper (202). Regarding flavonoids intake, the intakes of flavan 3-ols and isoflavones were significantly higher in men than in women. Even though, there is no data for MS, in Parkinson disease, it was found that people that consumed higher amounts of flavonoids has a lower risk of all-cause mortality and these results were more pronounced in men (203). However, there are no available recommendations about flavonoids either for the total population or sex-specific.

Phase angle in MS and the associations with nutrition and lifestyle factors

The median (IQR) phase angle of the total population was 5.35° (4.9 - 5.8°) and male as it can be assumed had significantly higher phase angle (P -value=0.006). Specifically, the mean phase angle for men was 5.6° and for women 5.3° . No study till now has investigated the phase angle in pwMS, consequently it is not known the common phase angle found in this population and if it differs from the general population. According to a known study by Kyle et al (2012) that took place in Geneva, Switzerland, the normal phase angle for men is $>5^{\circ}$ while for women $>4.6^{\circ}$ (204). On the other hand, a more recent systematic review and meta-analysis from 2019 classified the normal cut-offs for phase angle based on age and sex, showing that for adult men from 18 to 48 years old the phase angle stabilizes at 7.3 and for women at 6.4, while it decreases later in age, showing that the normal phase angle for adults may be much higher than defined in the past (205). Our population differs in age and pwMS above 48 years old are included, so the cut-off values of Kyle et al (2012), the pwMS included in this study are in the lower normal values of phase angle, while according to the systematic review, they may be below. Phase angle was divided in tertiles for the initial analysis. As shown, by the systematic review, in this analysis, too, people in the highest tertile of phase angle

were significantly younger than the pwMS at the lowest one. Indeed, the LASSO regression model showed that with the increase of age by a year, there is a decrease of about 0.0155° of phase angle and being female showed a negative association with phase angle. The best nutrient intake was noticed in the third tertile with the highest phase angle, but strangely the worst nutrient intake was reported for the middle tertile with phase angle from 5.1° to 5.6° , instead to the lowest one. As it was expected T3 had significantly higher protein intake, since low protein intake is associated with sarcopenia and reduced cell mass (206–208) and consequently low phase angle. As a result, it was also shown that pwMS at T1 had significantly more often low PA, while pwMS at the T3 had more often high PA. This was shown by the LASSO regression, too, but without results with clinical significance. In addition, pwMS with worse disability score (higher EDSS) had lower phase angle, which can be considered a consequence of lower muscle mass and movement based on the finding mentioned above. While there are no studies in pwMS, studies in different patient populations, such as patients with lumbar spinal stenosis (209), hemodialysis patients (210) and hospitalized patients (211) have found that higher functional disability is associated with lower phase angle. In this analysis, it was also found that better sleep quality was found in the tertile with the highest phase angle. While there are no studies, evaluating this connection, it is found that sleep quality is associated with muscle mass, so PA and therefore potentially phase angle. For example, in a study with older individuals with obesity (212). Moreover, vitamins from the complex B (thiamin, pantothenic acid, folic acid and vitamin B12) were significantly higher at T3. Even though there are no studies showing direct associations between vitamin B complex and phase angle, there are studies showing the important role vitamins B and cellular health, growth and function, more over poor energy metabolism because of vitamin B complex deficiencies may lead to lower cellular function and hence lower phase angle (213–215). Furthermore, zinc intake was significantly higher in T3, possibly because zinc is related to protein synthesis and muscle repair (216) and zinc deficiency is related to oxidative stress and inflammation (217) and therefore zinc intake could influence body composition and phase angle, since higher oxidative stress and inflammation are associated with lower phase angle, too (183). In this analysis, it was found that pwMS in T3 had significantly higher phosphorus intake. There is no study, showing a direct link of phosphorus intake and phase angle, however higher serum phosphorus, which is a proxy for phosphorus intake was associated with higher phase angle in patient populations (218), while another study showed a moderate correlation between phosphorus intake and higher phase angle (219), possibly because most of the high-protein foods contain high phosphorus, too (220). Moreover, iodine intake was

significantly higher in pwMS with higher phase angle. There is no evidence showing any relation between iodine and phase angle. Iodine could indirectly influence cellular function and phase angle, through its control over thyroid hormones (221,222). Flavonoids and specifically flavonones and anthocyanins were significantly higher in pwMS with higher phase angle. Flavonoids play an important role in reducing cell damage and are involved in cell signaling pathways related to oxidative stress, inflammation and apoptosis (223). An interesting result was that pwMS at the lowest tertile of phase angle had significantly higher 25(OH)D. It could be assumed that people with higher phase angle and possibly with lower disability and higher PA did not use vitamin D supplements, while people with worse progress may be more keen and more frequently suggested to be supplemented. Finally, an important association found with the LASSO regression model was the positive association between selenium intake and phase angle. No study till now has shown this association in pwMS or any other populations. The different indirect possible mechanisms for this association are the role of selenium in preservation of muscle mass and function (224) and its action as an antioxidant via selenoproteins against oxidative stress and inflammation (225) which have an effect on phase angle.

DII in MS and the associations with nutrition and lifestyle factors

Nutrients that are included in the calculation of DII showed significant differences across the tertiles, with nutrients that are supposed to decrease inflammation to be found in the highest intakes at lower tertile and lowest intakes in the highest tertile. Medi-lite score as it could be expected was significantly lower at the highest tertile for DII, since the Mediterranean diet is considered an anti-inflammatory diet, due to the variety of anti-inflammatory macro- and micro-nutrients included (226). Pantothenic acid, which is not included in the calculation of DII, was significantly lower at T3 compared to the other tertiles. No study has studied the effect of pantothenic acid intake on dietary inflammation in pwMS, but a study conducted in South Korea found a significant inverse relationship between pantothenic acid intake and C-reactive protein concentration, which is an inflammatory marker (227). Another micronutrient showing significantly higher intake at lower DII levels is potassium. A study investigating the association between salt and potassium intake in MS, showed that high salt intake induce pro-inflammatory cytokines and the development of MS, however the higher potassium intake did not show any significant correlation with the risk of MS, even though it was assumed that it may have an inverse role (228). In other studies, it is reported that potassium intake can modulate inflammation, such as the cases of inflammation in chronic kidney disease (229) and rheumatoid arthritis (230). Phosphorus intake was the lowest in T2 and the highest in T3,

suggesting that higher phosphorus intake is related to lower dietary inflammation. This comes in accordance with existing studies but not in pwMS. It has been suggested that increased phosphorus intake may influence inflammatory diseases in this case gingivitis by altering cytokine levels (231). Additionally, low copper intake was significantly linked with a more pro-inflammatory diet. Copper may have a double role in MS according to studies. Even though copper may have an antioxidant role, it is important to assess the ration between copper and zinc. Lower blood concentrations of zinc and a higher ratio of copper and zinc in pwMS may suggest a relationship between MS and oxidative stress, so the role of copper may depend on the concentration of zinc (232). Furthermore, the most anti-inflammatory diet reported the highest manganese intake. Manganese has a double role, too. Even though, it can have an anti-inflammatory role by reducing oxidative stress through its function as component of manganese superoxide dismutase (233), it is reported that manganese intake above nutritional adequacy could result in inflammatory biomarker production (234). A very interesting result from the LASSO regression is the negative association between 25(OH)D and DII. It was shown that for each 1 ng/ml increase of 25(OH)D, there is reduction in the DII of 0.011 units (P-value = 0.031), which could be an important decrease of diet-related inflammation. Higher vitamin D concentrations tend to decrease inflammation markers, but no study has shown the effect on DII in general and specifically in pwMS. People with chronic inflammatory diseases, such as chronic kidney disease, inflammatory bowel disease, nonalcoholic fatty liver disease and others tend to have lower 25(OH)D levels (235). As already mentioned vitamin D status is one of the most studied predictors of MS. Mendelian randomization analyses have suggested a causal relationship between low vitamin D levels and risk of MS, too (117), however most of the studies are focused on the preventive role of high vitamin D levels on MS (117), instead of the vitamin D concentrations and the inflammation in pwMS. A few available studies show an anti-inflammatory effect of dietary vitamin D3 and in general vitamin D supplementation in pwMS by potentially modulating the immune-inflammatory response (236,237). On the other hand, the needed dose is not defined and the studies involved a low sample size, as a result in a study by Lis et al (2024) conducted in Poland, it is concluded that the vitamin D dose supplemented may be too low to have beneficial effects against inflammation in pwMS (238).

Strengths

To the best of our knowledge this is the first study on pwMS with a high sample size evaluating a very high number of different exposome factors and specifically for nutrition and lifestyle making such an extended assessment of nutrient intake and indexes. Additionally, all the questionnaires

and indexes used are widely used and validated for the assessment of the specific variables. Quality of life was measured with a questionnaire (MSQoL) established specifically for this population (164) and inflammation related to diet was assessed with the use of DII, which may be a complex index considering the consumed amount of a variety of nutrients, food ingredients and flavonoids with a complicated calculation methodology, but it is recognized as one of the most accepted and precise tools for this purpose, since it is widely used in studies, it is validated in multiple populations with the use of inflammatory biomarkers and a comprehensive systematic review was conducted regarding the link between diet and inflammation (165). The DII has been used for this type of evaluation on pwMS, too (44), however mostly about the risk of developing DII. Another important strength of this study are the face to face interviews by trained dietitians/nutritionists that conducted in real time the anthropometrical measurements of the participants and conducted the dietary and lifestyle assessment through questions, in order to avoid the over- or under-estimation of food consumption or any other bias that could result because of misunderstanding of the lifestyle questionnaires (239). Furthermore, both 24h recalls and FFQs based on the Italian eating habits were administered to the participants, since these two diet evaluation methods alone have different advantages and disadvantages, but according to studies, the combination of them increase highly the precision of the estimation of usual dietary intakes (240). Also, the newest version of BIA was used for the assessment of body composition (AKERN BIA 101 BIVA® PRO device) (157), which allowed the accurate estimation of phase angle, showing the nutritional composition of the participants. Regarding the statistical methodology used, LASSO regression is a key strength of this analysis, since it allows to identify the most relevant predictors, while reducing the risk of overfitting and simplifying the models (241).

Limitations

One of the main limitations of this study is its design as it is a cross-sectional study, so exposures and outcomes are measured at the same moment in time without having the possibility to establish cause-effect relationships and incidence rates cannot be evaluated to measure the potential risk of developing new cases in this case according to nutrition and lifestyle habits. Moreover, cross-sectional studies may show snapshot bias, meaning that there may be fluctuations at the biomarkers, questionnaires and values reported, especially in this type of disease, where great changes may take place, when a patient is on the relapse phase or not (242). Another limitation of this study is that it was noticed that only people with relatively low EDSS were recruited and this could be considered rational considering that people with greater disability would be unwilling to

participate in the study and/or in difficulty to be assessed for all these parameters, particularly pwMS that did not live in Pavia and would need a caretaker. Finally, only pwMS from the provinces of Pavia and Milan were recruited and consequently, the results may not be representative for the MS population in Italy.

Conclusions and recommendations

This study represents the first comprehensive evaluation of nutrition, lifestyle and diet-related inflammation in people with RRMS living in the provinces of Pavia and Milan, Italy. The results highlight the complexity of the external exposome in MS and emphasize the significant role of lifestyle factors, particularly diet, in influencing QoL, functional capacity and inflammatory processes showing the crucial role of sex-specific differences. Phase angle, measured for the first time in this population, was positively associated with younger age, male sex, higher nutrient intake, greater physical activity and lower disability, with selenium intake showing a novel positive correlation. DII indicated that higher serum 25(OH)D levels were significantly linked to lower DII scores, suggesting that vitamin D status may modulate the inflammatory potential of the diet in pwMS. While the high sample size, detailed and validated assessment of dietary and lifestyle variables, advanced body composition analysis, and use of robust statistical approaches such as LASSO regression strengthen this study, certain limitations remain. The cross-sectional design and underrepresentation of participants with higher disability levels limit causal inferences and generalizability, highlighting the need for longitudinal research. Future studies including a wider range of disability could clarify how nutrition, lifestyle, phase angle and dietary inflammation affect MS outcomes over time. There is also a need to investigate personalized dietary interventions, particularly those targeting anti-inflammatory nutrients, while considering sex-specific differences in nutrition and lifestyle. Combining nutrition with tailored physical activity programs may promote muscle health, functional capacity, and overall well-being. Further exploration of phase angle as a biomarker of cellular health and disease progression is warranted. Finally, a better understanding of the role of nutrition in MS could inform evidence-based dietary guidelines and holistic approaches addressing sleep, mental health, diet and physical activity may provide practical strategies for improving long-term outcomes in pwMS through educational and lifestyle programs.

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