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**Experimental Thesis**

# Evaluation of the relationship between Diet and risk of Multiple Sclerosis

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## Abbreviation

**1, 25(OH) 2D** - 1, 25-Dihydroxyvitamin D

**ACTH** - Adrenocorticotropic Hormone

**aOR** - Adjusted Odds Ratio

**BBB** - Blood-Brain Barrier

**BMI** - Body Mass Index

**CI** - Confidence Interval

**CNS** - Central Nervous System

**DHA** - Docosahexaenoic Acid

**DMTs** - Disease-Modifying Treatments

**EAE** - Experimental Autoimmune Encephalomyelitis

**EBV** - Epstein Barr Virus

**ENS** - Enteric Nervous System

**EnvIMS** - Environmental Risk Factors in MS

**EPA** - Eicosapentaenoic Acid

**FFQ** - Food Frequency Questionnaire

**GALT** - Gut-Associated Lymphoid Tissue

**GI** - Gastrointestinal

**HLA** - Human Leukocyte Antigen

**HR** - Hazard Ratio

**ICD-10** - International Classification of Diseases, Tenth Revision

**HHV6** - Human Herpes Virus type 6

**LPS** - Lipopolysaccharides

**LXR** - Liver X Receptor

**MDS** - Mediterranean Diet Scale

**MD** - Mediterranean Diet

**MDS** - Mediterranean Diet Score

**MHC** - Major Histocompatibility Complex

**MRI** - Magnetic Resonance Imaging

**MS** - Multiple Sclerosis

**NF-kB** - Nuclear Factor kappa B

**NHS** - National Health Service

**OR** - Odds Ratio

**PNS** - Peripheral Nervous System

**PPAR** - Peroxisome Proliferator-Activated Receptor

**PUFAs** - Polyunsaturated Fatty Acids

**ROS** - Reactive Oxygen Species

**SCFAs** - Short-Chain Fatty Acids

**SREBP-1c** - Sterol Regulatory Element-Binding Protein 1c

**Th1, Th2, Th17** - Types of T-helper cells

**UK Biobank** - United Kingdom Biobank

# 1. Summary

## 1.1. Rationale of the Study

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS), predominantly affecting young adults, especially females. The increasing prevalence of MS highlights the need to understand its causes. Genetic, environmental, and immune factors contribute to MS susceptibility, but mechanisms remain unclear.

Dietary habits and lifestyle choices are modifiable risk factors in autoimmune conditions. However, the relationship between diet and MS is underexplored. This study investigates how dietary patterns might influence MS risk, offering new avenues for prevention and management.

Moderate consumption of oily fish and weekly alcohol intake are associated with a reduced risk of MS. There is also a non-significant trend toward a protective effect from greater adherence to the Mediterranean diet. These findings, while needing further validation, provide a promising direction for research and dietary recommendations for MS prevention.

## 1.2. Planning of the Study

Using UK Biobank data, this study explores the link between diet, lifestyle, and MS risk. Data from a food frequency questionnaire (FFQ) completed at enrollment were used to compare the habits of individuals who developed MS with those who did not. A subset analysis using 24-hour recall data provided deeper insights, enhancing reliability.

## 1.3. Results

The study indicates that moderate intake of oily fish and weekly alcohol consumption are protective factors against MS. There is also a non-significant trend suggesting increased adherence to the Mediterranean diet may provide protection.

## 1.4. Conclusion

This study represents a pioneering effort to elucidate the role of diet in MS etiology using the comprehensive data available from the UK Biobank. The initial findings support the notion that dietary modifications could be a viable strategy for reducing MS risk. Further research is warranted to confirm these results and to explore the underlying mechanisms, ultimately aiming to inform dietary guidelines and public health strategies for MS prevention.

# 2. Introduction

## 2.1. Multiple Sclerosis Definition and Epidemiology

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS), characterized by inflammation and axonal demyelination (McGinley, Goldschmidt et al., 2020). The global prevalence and incidence of MS have shown an increasing trend, with estimates in 2020 showing rates of 35.9 per 100,000 and 2.1 per 100,000, respectively. However, regional data vary significantly due to genetic and environmental factors, as well as inconsistencies in medical data collection. The highest prevalence is observed in the Americas and Europe, while Latin America reports the lowest (Negrotto and Correale, 2018).

MS, along with other autoimmune diseases of the nervous system like neuromyelitis optica spectrum disorder (NMOSD) and autoimmune encephalitis, exhibits a higher prevalence among females, highlighting a common gender predilection in these conditions (Altintas, Dargvainiene et al., 2020). Retrospective studies consistently demonstrate that women are disproportionately affected by MS, with prevalence rates reported to be two to three times higher compared to men (Orton, Herrera et al., 2006). This gender disparity underscores the significant impact of sex-related factors in the epidemiology of MS.

The onset of MS typically occurs between the ages of 20 and 40 years, representing the most common period for disease onset, although manifestations can occur at any age beyond adolescence (Hosseinzadeh, Baneshi et al., 2019).

## 2.2. Clinical Manifestation and Diagnosis

Multiple sclerosis (MS) is characterized by a diverse spectrum of symptoms, reflecting its multifocal and inflammatory nature within the central nervous system (CNS). Clinical presentations vary widely among individuals but commonly include neurological deficits such as blurred vision with pain (optic neuritis), focal sensory disturbances, extremity weakness, hearing loss, and facial sensory disturbances (McGinley, Goldschmidt et al., 2020).

Optic neuritis, often presenting with unilateral blurred vision and eye pain exacerbated by movement, is a hallmark of MS. It results from inflammation of the optic nerve, leading to demyelination and impaired visual transmission. Other sensory disturbances may include tingling sensations, numbness, or hypersensitivity in limbs or facial regions, indicative of sensory pathway involvement due to demyelinating lesions in the spinal cord or brain (McGinley, Goldschmidt et al., 2020).

The diagnosis of MS relies on a combination of clinical evaluation, neuroimaging findings, and laboratory tests to confirm the presence of CNS demyelination and exclude alternative diagnoses. Magnetic resonance imaging (MRI) plays a crucial role in visualizing MS-related lesions in the brain and spinal cord, demonstrating characteristic T2-hyperintense lesions that indicate areas of demyelination and inflammation (McGinley, Goldschmidt et al., 2020).

Diagnostic criteria emphasize "dissemination in space" and "dissemination in time" to differentiate MS from other demyelinating conditions. Dissemination in space refers to the presence of lesions in distinct anatomical locations within the CNS, either by clinical presentation involving different CNS areas or by MRI showing multiple T2-hyperintense lesions. Dissemination in time involves the progressive development of new lesions over time, indicating ongoing disease activity and supporting the diagnosis of MS (McGinley, Goldschmidt et al., 2020).

MRI not only aids in diagnosis but also serves as a critical tool for monitoring disease progression and treatment response in MS patients. Serial imaging allows clinicians to track lesion development, assess treatment efficacy, and adjust management strategies accordingly. This proactive approach enhances patient care by facilitating early intervention and optimizing long-term outcomes (McGinley, Goldschmidt et al., 2020).

MS presents with a wide array of clinical manifestations reflecting its heterogeneous nature. Optic neuritis, sensory disturbances, motor deficits, and fatigue are common symptoms, underscoring the complexity of CNS involvement in MS pathogenesis. Accurate diagnosis relies on integrating clinical findings with advanced imaging techniques that highlight lesions' spatial and temporal dissemination. Continued advancements in diagnostic criteria and imaging technology promise to

further refine MS diagnosis and management strategies, ultimately improving patient outcomes and quality of life.

### 2.3. Types

Understanding the types of MS helps determine the treatment and prognosis of the disease. The disease can be classified into seven types based on its course:

- Relapsing-remitting (RR) MS, affecting 70-80% of patients initially, features periodic relapses with new or recurrent neurological symptoms lasting 24-48 hours, followed by partial or complete recovery.
- Primary progressive MS affects 15-20% of patients and is characterized by gradual worsening without distinct relapses or remissions.
- Secondary progressive (SP) MS shows steady disease progression with or without occasional relapses.
- Progressive-relapsing (PR) MS, affecting about 5%, involves worsening disability with superimposed relapses.
- Clinically isolated syndrome (CIS) is the earliest stage or precursor to MS.
- Fulminant MS is a severe form with rapid progression towards disability.
- Benign MS is a mild form with minimal disability over many years (Tafti, Ehsan et al., 2024).

### 2.4. Myelin structure and Function

The study of myelin spans centuries, beginning with Van Leeuwenhoek's discovery of myelinated fibers in 1717. In 1854, Rudolf Virchow identified the chemical composition of myelin and coined its name (Kister and Kister, 2023).

The nervous system is composed of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain, spinal cord, and certain nerves with myelin from oligodendrocytes, which myelinate multiple axons but only one segment per axon based on size. The PNS consists of other nerves with myelin from Schwann cells, which myelinate single axons wider than 1 μm. The Obersteiner-Redlich zone demarcates the boundary between CNS and PNS

myelin. Differences in lipid and protein composition explain disease preferences. Astrocytes and microglia aid in myelin development and maintenance; microglia clear damaged myelin, and Schwann cells assist after nerve injury. Understanding these processes is crucial for studying diseases such as multiple sclerosis and peripheral neuropathies (Kister and Kister, 2023).

In multiple sclerosis, the loss of myelin plays a significant role both clinically and pathologically. Myelin is produced by oligodendrocytes in the CNS and Schwann cells in the PNS. It wraps around axonal segments, creating internodes. The primary function of myelin is to facilitate impulse propagation along axons in both the CNS and PNS. Myelin comprises a high lipid-to-protein ratio and accounts for about 70-85% of the dry weight in both the CNS and PNS. The most abundant lipids present in myelin are cholesterol, glycolipids (such as cerebroside and cerebroside sulfate), and ethanolamine glycerophosphatides. CNS myelin contains more glycolipids and less sphingomyelin compared to myelin in the PNS (Oudejans, Luchicchi et al., 2021).

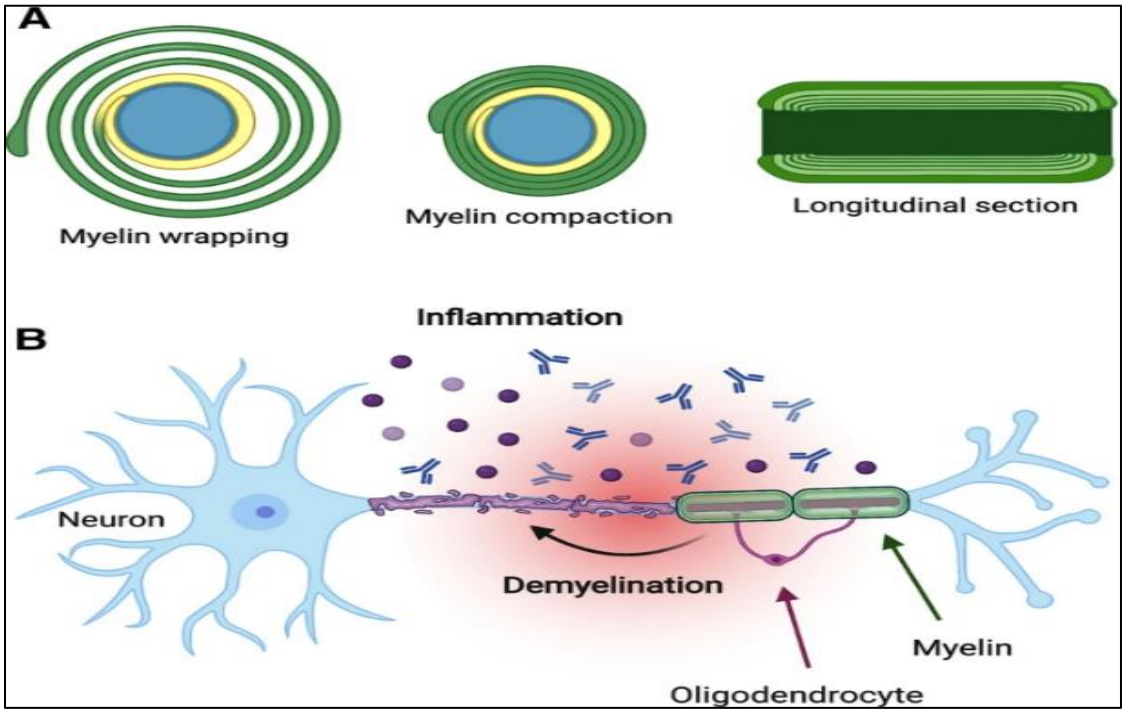


*Figure 1 Myelin formation and degradation. (Martinsen and Kursula et al.2022)*

The structure of myelin, extensively studied through electron microscopy, reveals a multilayered arrangement characterized by alternating electron-dense and light layers known as the major dense

line and intraperiod line. These layers result from compaction between membranes, occurring at a periodicity of approximately 12 nm. Myelin segments attach to axons through terminal loops containing cytoplasm. The myelin sheath includes distinct regions: the paranode (~4 µm), juxtaparanode (10-15 µm), and nodal region (0.8-1.1 µm), where axon diameter is reduced. Astrocytic processes cover nodes of large fibers, forming microvilli-like contacts with outer paranodal loops of oligodendrocytes (Stadelmann, Timmler et al., 2019).

### 2.5. Pathogenesis of Multiple Sclerosis

The exact pathological process of how MS develops and progresses remains unknown. It is understood to be a complex condition influenced by immune, genetic, and environmental factors (Tsunoda & Fujinami, 2002; Waubant, Lucas et al., 2019).

MS is characterized by autoreactive immune cells crossing the blood-brain barrier (BBB) to attack the central nervous system (CNS). Physiologically, central tolerance mechanisms remove autoreactive immune cells during development in the thymus or bone marrow. Furthermore, peripheral tolerance mechanisms prevent those that escape this process and enter circulation from causing disease. Peripheral tolerance is impaired due to malfunction of regulatory T cells or resistance of autoreactive T cells to suppression. B cells also play a role, as antibody-secreting cells derived from B cells within the CNS contribute to excessive immunoglobulin production, which is a hallmark of MS (Dighriri, Aldalbahi et al., 2023).

In MS, inflammation of the CNS white and grey matter results from focal immune cell infiltration and cytokine activity. CD4+ T cells and adaptive immune responses play key roles in disease initiation and progression. Antigen-presenting cells interact with T lymphocytes to produce cytokines such as IL-12, IL-23, and IL-4, driving CD4+ T cell differentiation into Th1, Th2, and Th17 phenotypes. Th1 cells release IFNγ and TNF-α, promoting inflammation, while Th2 cells secrete IL-4 and IL-13, which activate M2 macrophages to reduce inflammation. Th17 cells produce IL-17, IL-21, IL-22, and IL-26, contributing to MS inflammation and disease complexity (Ghasemi, Razavi et al., 2017).

The first MS-associated genetic locus, discovered in 1972 and located in the HLA class I region on chromosome 6, significantly contributed to MS research. Other studies have shown that several HLA (Human Leukocyte Antigen) class I and II alleles are linked to increased or decreased MS risk. Specifically, the HLA DRB1*1501 allele demonstrates a strong association with nearly triple the risk of MS. Recent genome-wide studies have identified multiple independent MS risk variants within the major histocompatibility complex (MHC) region, which alone explains about 20% of MS inheritance. The MHC region contains over 200 genes involved in T cell regulation and immune processes, indicating its immune-mediated role in MS. Studies indicate that certain MS-associated variants in the MHC region affect peptide-binding, thereby influencing T-cell responses crucial in MS pathogenesis (Ferrè, Filippi et al., 2020).

Environmental factors such as Epstein Barr virus (EBV), Human herpes virus type 6 (HHV6), mycoplasma pneumonia, smoking, vitamin deficiencies, diet, and UV radiation are linked to MS. These factors can trigger immune responses that lead to myelin sheath lesions. Smoking increases Nitric oxide (NO) and Carbon monoxide (CO), causing oxidative damage and inflammation, while vitamin D and B12 influence immune function and myelin synthesis. Sunlight promotes vitamin D production and may modulate MS through anti-inflammatory effects. Dietary patterns also affect MS risk; high fish intake may lower risk, while animal fat intake may increase it (Ghasemi, Razavi et al., 2017).

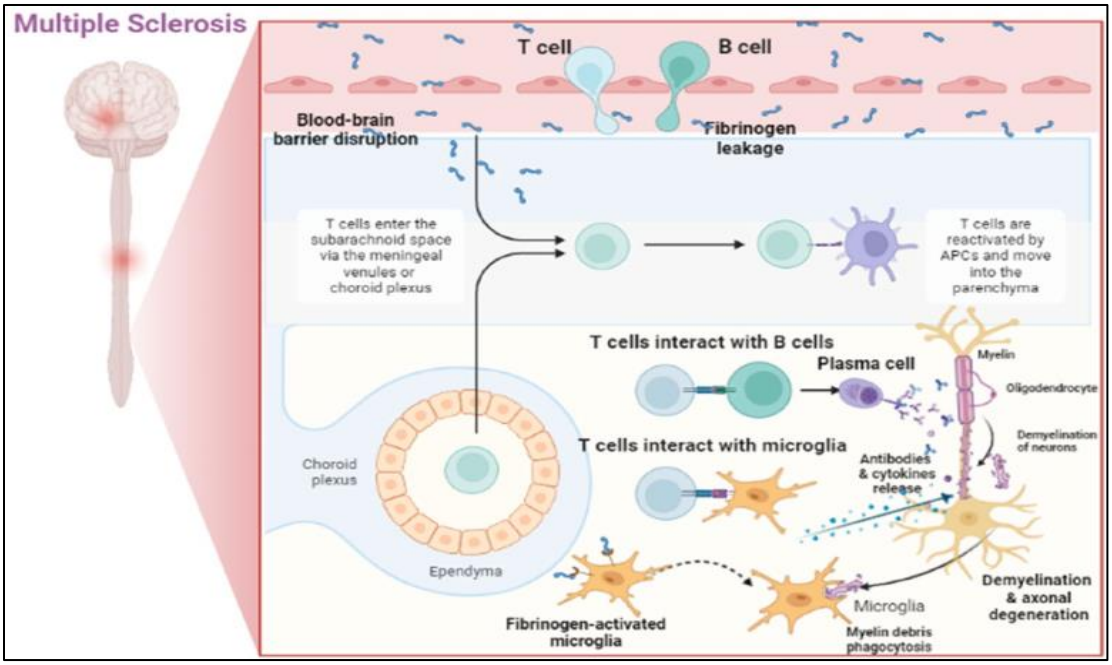


*Figure 2 Pathophysiology of Multiple sclerosis. (Al-Kuraishy, Al-Gareeb et al.2023)*

Multiple Sclerosis (MS) can be classified into four distinct pathological types based on specific cellular mechanisms and patterns of damage. Type 1 MS primarily involves demyelination driven by cell-mediated immune responses, prominently featuring the involvement of macrophages. In Type 2 MS, both cell-mediated immunity and humoral components such as antibodies and complement proteins contribute to tissue damage. Type 3 MS centers around dysfunction and apoptosis of oligodendrocytes, primarily affecting myelin proteins. Lastly, Type 4 MS is characterized by oligodendrocyte injury akin to that caused by infectious agents or toxins, which does not stem from autoimmune processes. This classification is critical for guiding treatment strategies tailored to each subtype and deepening our understanding of the diverse etiological factors contributing to MS pathology (Shah, Panchal et al.2023)

### 2.6. Gut microbial Axis

The intestinal microbiota has been implicated as a trigger for the onset of Multiple Sclerosis (MS), as demonstrated in experimental models of MS and experimental autoimmune encephalomyelitis (EAE) (Wekerle, 2017). The term "gut-brain axis" describes the bidirectional communication pathway between the central nervous system (CNS) and the external environment. This axis comprises the microbiota, intestinal barrier, and enteric nervous system (ENS). The microbiota

regulates the normal physiology of the gastrointestinal (GI) system, the barrier controls the movement of food and microbial products into the organism, and the ENS coordinates both parasympathetic and sympathetic innervations of the GI system (Parodi and Kerlero De Rosbo, 2021).

The gut environment contributes to the development of MS by increasing intestinal permeability, which disrupts the intestinal epithelial barrier. This disruption allows microbial translocation from the gut into the lamina propria, triggering local inflammation. This inflammatory response activates encephalitogenic T cells, which migrate to the CNS, thereby initiating inflammatory damage, demyelination, and axonal loss. Moreover, neuronal inflammation in MS may also arise from altered blood-brain barrier permeability, leading to microglial activation, inflammatory cell infiltration, and signaling pathways that further exacerbate intestinal barrier permeability (Parodi and Kerlero De Rosbo, 2021).

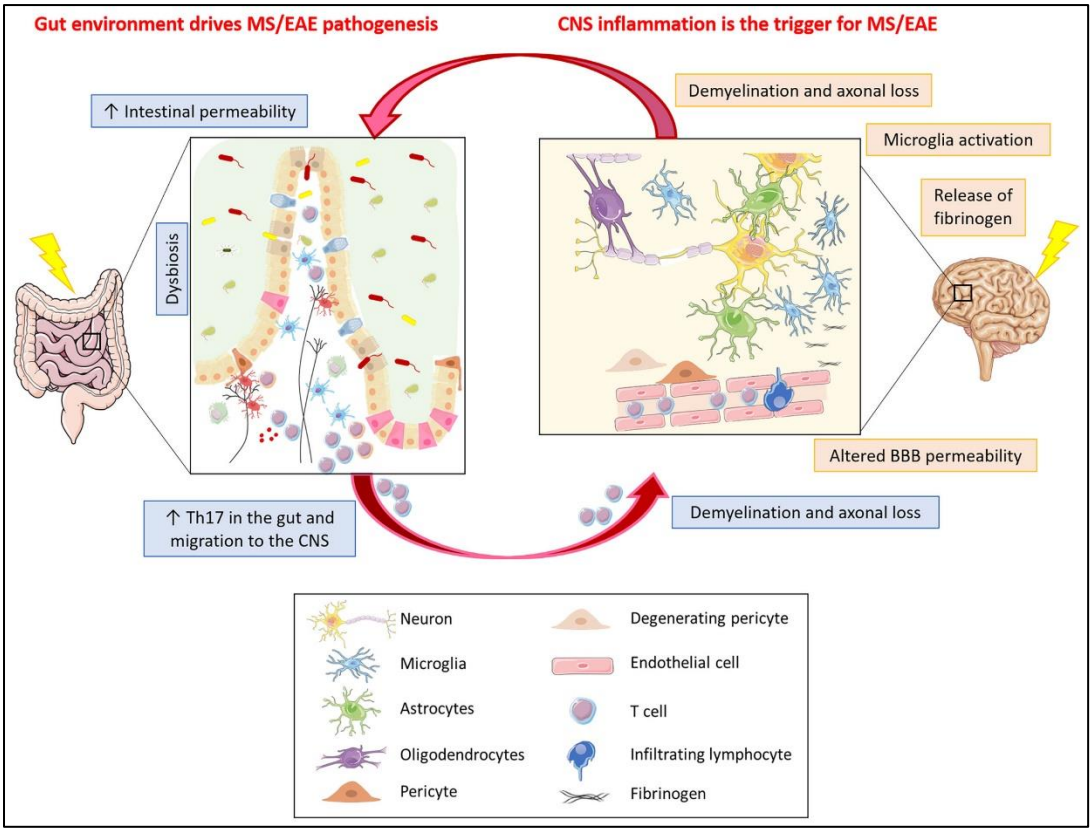


*Figure 3 Relationship between gut environment and CNS inflammation. (Parodi and Kerlero De Rosbo 2021)*

The gut bacterial composition in both healthy individuals and those with Multiple Sclerosis (MS) predominantly consists of Firmicutes and Bacteroidetes phyla. Within the Firmicutes phylum, many species are known for producing short-chain fatty acids (SCFAs) such as butyrate. Butyrate exerts anti-inflammatory effects on epithelial cells, reinforcing the intestinal barrier and preventing the entry of external or microbial antigens into the systemic immune system (Del Negro, Pez et al., 2023).

The gut microbiota plays a critical role in the communication between the gut and brain, with emerging research highlighting its potential as a therapeutic target for CNS diseases like MS. In healthy individuals, SCFAs produced by gut microbiota help maintain the integrity of the blood-brain barrier (BBB). However, dysbiosis or alterations in gut microbiota can reduce SCFA production, leading to BBB dysfunction. This breakdown can trigger systemic inflammation, prompting immune cells to produce cytokines that contribute to central nervous system (CNS) demyelination in MS (Zhang, Wang et al., 2024).

Numerous studies indicate that microbial dysbiosis impacts autoimmune diseases by altering the composition of specific bacterial species, thereby promoting intestinal inflammation, influencing gut-associated lymphoid tissue (GALT), and increasing intestinal permeability (leaky gut) (Calvo-Barreiro, Eixarch et al., 2018). Modulating the gut-brain axis (GBA) through dietary interventions holds promise in influencing MS pathogenesis by regulating the body's inflammatory response. Evidence suggests that nutrition and various diets significantly affect the development and progression of MS (Jayasinghe, Prathiraja et al., 2022).

### 2.7. Treatment of Multiple Sclerosis

The treatment of multiple sclerosis (MS) employs a comprehensive approach tailored to each patient's specific needs. Corticosteroids are the first-line treatment for severe relapses, effectively reducing inflammation and promoting rapid recovery. In cases where corticosteroids are ineffective, adrenocorticotropic hormone (ACTH) may be administered. Plasmapheresis is another option aimed at removing MS-related antibodies and proteins when initial treatments fail to yield adequate results.

Disease-modifying treatments (DMTs) play a crucial role by reducing the risk of relapses, MRI-detected disease activity, and accumulation of symptoms. Early initiation of DMTs is essential to delay neurodegeneration and prevent disability progression. In addition to pharmacological interventions, general measures such as exercise and physical therapy are integral components of MS management.

Symptomatically, spasticity is managed pharmacologically using medications like Baclofen and tizanidine. Non-pharmacological approaches such as stretching exercises, electrical stimulation, and cryotherapy also contribute to spasticity management. Depression, commonly associated with MS, is addressed through antidepressants and cognitive behavioral therapies.

Ongoing advancements in MS treatment include the investigation of immunomodulatory drugs like rituximab and simvastatin, showing promising outcomes in current clinical trials. Continued research aims to refine therapeutic approaches further, ultimately improving outcomes and enhancing the quality of life for individuals living with MS (Shah, Panchal et al., 2023).

### 2.8. Effect of Diet in Multiple Sclerosis Development

Increasing evidence suggests that neurodegeneration plays a critical role in the pathogenesis of multiple sclerosis (MS), regardless of its clinical presentation (Sandi, Fricska-Nagy et al., 2021). Oxidative stress and mitochondrial dysfunction are key contributors to neurodegenerative diseases, as mitochondria, the primary source of intracellular reactive oxygen species (ROS), are particularly vulnerable to oxidative damage (Guo, Sun et al., 2013).

Dietary antioxidants have shown promise in reducing oxidative stress, potentially protecting against chronic demyelination and neuronal damage in MS. Omega-3 polyunsaturated fatty acids (PUFAs), abundant in foods like fish, nuts, and seeds, possess antioxidant properties and have been associated with mitigating neurodegeneration in MS. Consumption of PUFAs may offer protective effects against demyelination, highlighting their beneficial role (Katz Sand, 2018).

Omega-3 PUFAs are renowned for their anti-inflammatory properties and are found in various sources such as olive oil, rapeseed oil, walnuts, flaxseed, and oily marine fish. Studies have also identified natural compounds like oleanolic acid and erythrodiol, which exhibit protective effects in experimental MS models (EAE) by reducing inflammatory cytokines and disease severity. Olive oil is currently recommended for prophylactic use in MS patients, although further research is needed for consistent outcomes and broader application (Angeloni, Malaguti et al., 2017).

Vitamin D, obtained primarily through sunlight exposure, diet, and supplementation, plays a crucial role in immune function. The biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)2D], is essential for lymphocyte activation, proliferation, T-helper cell differentiation, and immune response regulation (Mora, Iwata et al., 2008). Observational studies suggest that adequate vitamin D levels may lower MS risk and influence disease progression, but definitive conclusions are hindered by study limitations, necessitating further investigation (Sintzel, Rametta et al., 2018).
Recent evidence underscores the impact of serum vitamin D levels on MS risk and disease activity. While observational studies historically supported the benefits of higher vitamin D levels, attributing a specific effect to vitamin D on MS remains challenging due to research constraints (Sintzel, Rametta et al., 2018).

Dietary antioxidants like curcumin and various fatty acids have been investigated for their potential to modulate immune cell activation and reduce inflammation and oxidative stress. These mechanisms are crucial in preventing chronic demyelination and axonal damage observed in MS (Bagur, Murcia et al., 2017).

Nutritional components and specialized diets can modulate inflammatory pathways. Saturated and trans fatty acids, along with lipopolysaccharides (LPS), may promote inflammation, while calorie restriction, polyphenols, and Omega-3 PUFAs tend to exert anti-inflammatory effects (Esposito, Maniscalco et al., 2021).

Polyphenols, abundant in vegetables, cereals, legumes, fruits, and beverages like tea and coffee, possess diverse properties including anti-inflammatory, immune-modulatory, and antioxidant

effects. Resveratrol, a type of polyphenol, is recognized for its neuroprotective effects, although there are reports suggesting it may exacerbate experimental MS-like diseases (Sato, Martinez et al., 2013).

Dietary factors can influence the production of inflammatory molecules such as tumor necrosis factor, interleukins, MMP9, prostaglandins, and leukotrienes, thereby impacting inflammation and oxidative stress in the body (Esposito, Maniscalco et al., 2021). Antioxidants such as polyphenols and carotenoids found in fruits and vegetables have been explored for their potential benefits in MS by reducing oxidative stress and mitigating inflammation and neuronal damage, indicating a potential protective role against MS (Jacobs, Gross et al., 2009).

Observational studies suggest a link between diet and MS, but findings are inconsistent regarding whether diet directly causes MS. Mendelian randomization studies indicate that increased consumption of cereals, vitamin C, folic acid, and fish oil, coupled with reduced intake of pork and alcohol, may potentially lower MS risk and severity. These insights can inform the development of dietary strategies for both preventing and managing MS (Wang, Moustaid-Moussa et al., 2014).

### 2.9. Mediterranean Diet and Multiple Sclerosis (MS)

Diet has emerged as a significant area of interest in multiple sclerosis (MS) research, with numerous studies exploring dietary patterns and their potential association with MS. Observational research suggests individuals with MS often follow less healthy or more pro-inflammatory diets compared to control groups (Wahls, 2022; Keykhaei, Norouzy et al., 2012).

In nutritional epidemiology, there's a growing recognition of the importance of pattern analysis over studying individual nutrients. This approach acknowledges that food combinations can have synergistic or antagonistic effects that extend beyond the impact of single dietary components (Frank B, 2022; Dinu, Pagliai et al., 2018).

Recent pooled analyses strongly support the hypothesis that the Mediterranean diet (MD) may help prevent chronic diseases, including multiple sclerosis (MS) (Esposito, Maniscalco et al., 2021).

Originating from Southern European countries, parts of Africa, and the Middle East, the MD emphasizes fruits, vegetables, nuts, legumes, whole grains, and fish, with moderate consumption of dairy and limited meat. Olive oil and moderate alcohol, such as red wine with meals, are also encouraged (Sotos-Prieto, Del Rio et al., 2022).

The Mediterranean diet is more than a collection of healthy foods; it embodies a cultural archetype that includes how foods are selected, prepared, and shared, along with other lifestyle practices. UNESCO recognized the Mediterranean diet as intangible cultural heritage in 2010 (Dernini, Berry et al., 2015). It was also highlighted in the 2015–2020 Dietary Guidelines for Americans as a health-promoting dietary pattern (Tagtow, Rahavi et al., 2022).

Alfredsson and colleagues found that adherence to the Mediterranean diet was associated with a lower risk of developing MS compared to a Western-style diet. However, they did not observe significant associations with vegetarian/vegan diets or diets with a low glycemic index (Alfredsson, Olsson et al., 2023). Additionally, following a healthy eating pattern has been linked to a 25% reduction in the risk of clinically isolated MS compared to a Western-style diet (Black, Rowley et al., 2019).

Historical documentation highlights the specific dietary habits of Mediterranean populations, shaped by geographical location and climate. Evidence from ancient times to recent history underscores the integration of certain foods into everyday diets, distinguishing Mediterranean dietary practices from those in other regions (Cristiani, Radini et al., 2016).

The Seven Countries Study by Ancel Keys, focusing on Southern Italy, was pivotal in demonstrating the potential health benefits of Mediterranean diets, despite some initial overestimations in hypotheses like the lipid hypothesis (Keys, 1995). Numerous studies since then have consistently shown that adhering to the Mediterranean diet significantly benefits cardio-metabolic health, reduces the risk of neurodegenerative diseases, and lowers the incidence of certain cancers (Dinu, Pagliai et al., 2018).

Various indices, such as the Mediterranean Diet Scale (MDS) and Mediterranean Diet Score (MDS), have been developed to measure adherence to the Mediterranean diet. These indices consider key characteristics of the diet to quantify adherence levels, despite inconsistencies in their components and scoring methods (Aoun, Papazian et al., 2019).

The mechanisms underlying the Mediterranean diet's beneficial effects include lipid-lowering actions, antioxidant and anti-inflammatory properties, modulation of cancer-related molecules, inhibition of nutrient-sensing pathways, and the production of metabolites by gut microbiota that impact metabolic health (Tosti, Bertozzi et al., 2018).

While research on diet and MS often focuses on specific nutrients like vitamin D and polyunsaturated fatty acids (PUFAs), broader studies across neurodegenerative diseases suggest potential synergistic benefits from comprehensive dietary patterns. Exploring these synergies could enhance our understanding of how diet influences MS onset and progression (Qu, Black et al., 2022).

More research is needed to explore the full potential of nutrients and dietary patterns in influencing MS, emphasizing antioxidants and anti-inflammatory foods. Integrating findings across various neurodegenerative diseases could provide valuable insights into shared mechanisms and optimize dietary recommendations for MS management and prevention (Qu, Black et al., 2022).

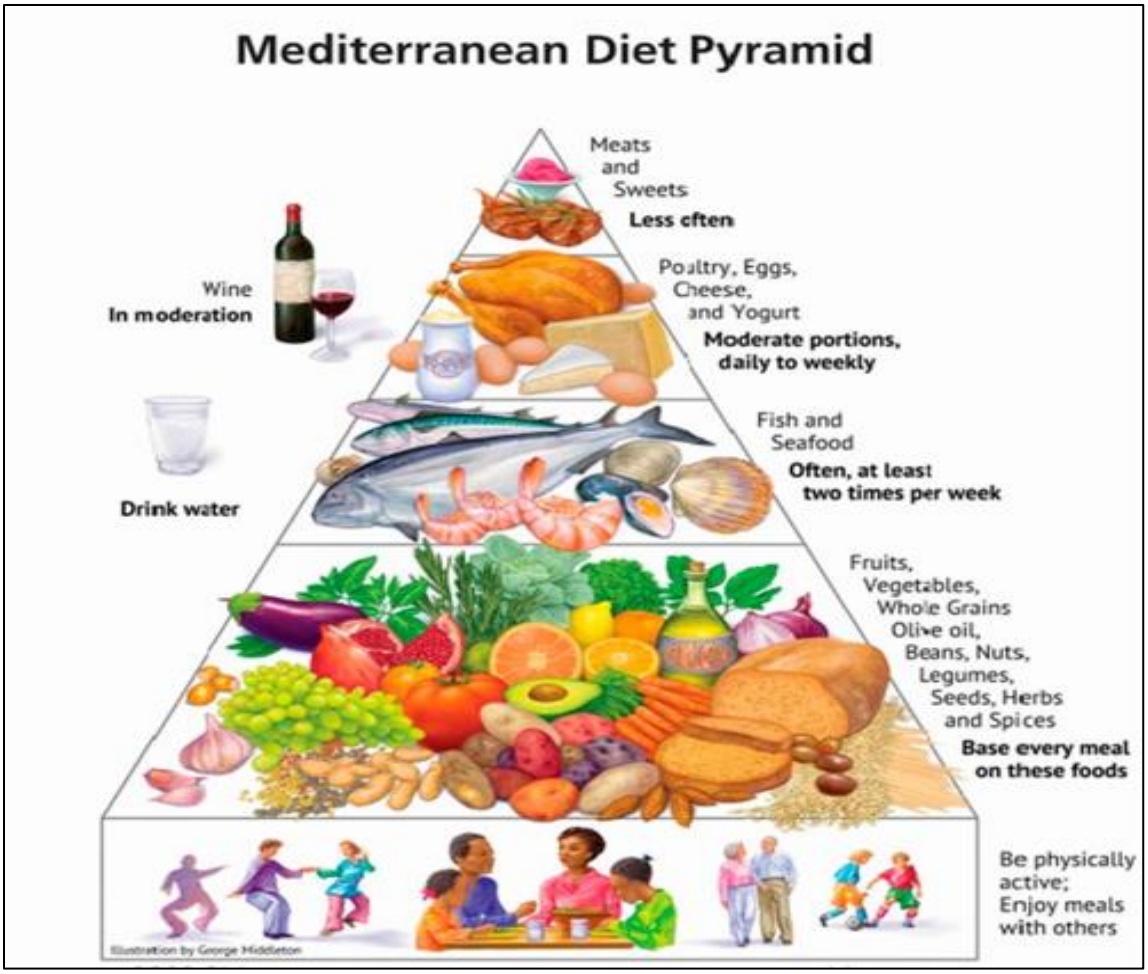


*Figure 4 The Mediterranean Diet Pyramid with olive oil as an important nutritional fat source. (Mazzocchi, Leone et al.2022)*

# 3. Objective

The aim of this study is to analyze the relationship between diet and the risk of multiple sclerosis (MS) onset by using data from the UK Biobank to measure the association between adherence to the Mediterranean Diet (MD) and MS risk.

## 3.1. Specific Objectives

**3.1.1. Objective 1:** Assess dietary patterns and adherence to the Mediterranean Diet among participants in the UK Biobank.

- **Utilize dietary intake data:** Comprehensive dietary intake data from the UK Biobank, including detailed information on food frequency and portion sizes, will be utilized to assess participants' adherence to the Mediterranean Diet.

- Develop Mediterranean Diet adherence score: The Mediterranean Diet adherence score will be calculated using validated scoring systems the Mediterranean Diet Score (MDS) This score will consider consumption of key components such as vegetables, legumes, fruits and nuts, cereals, fish and seafood, monounsaturated to saturated fat ratio, dairy products, meat, and alcohol.

**3.1.2. Objective 2:** Evaluate the association between adherence to the Mediterranean Diet and the risk of MS onset.

- **Statistical analyses:** Advanced statistical techniques, including Cox proportional hazards models will be employed to evaluate the association between adherence to the Mediterranean Diet and MS risk.

- **Control for confounding factors:** Potential confounding factors such as age, sex, socioeconomic status (measured by the Townsend Deprivation Index), smoking status (never, current, past), physical activity levels, (moderate, vigorous), and BMI categories (normal weight, overweight, obese) will be carefully controlled for in the analyses.

**3.1.3. Objective 3:** Provide recommendations for dietary guidelines aimed at MS prevention based on the findings.

- **Translate research findings into practical recommendations:** The research findings will be translated into evidence-based dietary recommendations aimed at reducing the risk of MS onset. Recommendations may include specific dietary modifications to enhance adherence to the Mediterranean Diet or other beneficial dietary patterns.

- **Highlight dietary interventions**: Emphasize the potential impact of dietary interventions in MS prevention and management strategies. Considerations may include public health policies, educational initiatives, and individual counseling aimed at promoting healthy dietary habits.

By achieving these objectives, the study aims to significantly contribute to the understanding of how dietary patterns, particularly adherence to the Mediterranean Diet, influence the risk of developing multiple sclerosis. The findings will provide valuable insights for clinicians, policymakers, and individuals at risk of MS, guiding the development of targeted interventions to promote optimal dietary practices for MS prevention and management.

# 4. Materials and Methods

## 4.1. Cohort Selection

This study utilized data from the UK Biobank, a large-scale prospective cohort study aimed at investigating the complex interactions between genetic predisposition, environmental exposures, lifestyle factors, and various health outcomes. The UK Biobank cohort initially recruited over 500,000 participants aged 40-69 years from across the United Kingdom between 2006 and 2010. Participants were selected from NHS patient registers and invited to participate via postal invitation to attend one of the UK Biobank assessment centers. These centers were strategically located across England, Scotland, and Wales, facilitating broad representation of the UK population.

At the assessment centers, participants underwent a detailed assessment process that included completing a comprehensive touchscreen questionnaire. This questionnaire collected a wide array of data, encompassing sociodemographic information (e.g., age, sex, and ethnicity), lifestyle behaviors (e.g., smoking status, physical activity levels), medical history (including self-reported conditions), dietary habits, and general health status. The comprehensive nature of these assessments allowed for a robust exploration of potential risk factors and health outcomes, including the onset of multiple sclerosis (MS).

### 4.1.1. Exclusion Criteria

To ensure the validity and focus of the study on incident MS cases and dietary patterns, several exclusion criteria were applied:

- **Prior Diagnosis of MS:** Participants with a documented diagnosis of multiple sclerosis (MS) at the time of enrollment or during the study period were excluded. This criterion aimed to focus on incident cases of MS and avoid bias from prevalent cases.
- **Withdrawal from the Study:** Participants who withdrew consent or discontinued their participation in the UK Biobank study were excluded from the analysis to maintain cohort integrity.

- **Missing Dietary Information:** Participants with incomplete or insufficient dietary data from the Oxford WebQ questionnaire were excluded from specific analyses where dietary patterns were the primary focus.
- **Self-Reported MS Diagnosis:** Participants who self-reported a diagnosis of MS during the study period but lacked confirmed medical records were excluded to ensure accuracy and reliability in MS case ascertainment.

These criteria were essential in refining the study cohort to focus on incident MS cases and ensure the reliability and validity of associations between dietary factors, lifestyle behaviors, and MS risk.

### 4.2. Dietary Data Collection

Dietary intake data in this study were collected using the Oxford WebQ, an online dietary assessment tool specifically designed for large-scale epidemiological studies. The Oxford WebQ questionnaire was administered to participants in four cycles between February 2011 and June 2012, capturing detailed information on their food and beverage consumption over a 24-hour period preceding each assessment. This tool is renowned for its user-friendly interface and robust data collection capabilities, covering 21 food groups and offering over 200 predefined food options. Participants selected their food choices from these options, minimizing the need for open-ended responses and ensuring standardized data collection across the cohort.

Following completion of the questionnaire, participants had the opportunity to review and make amendments to their dietary entries on a summary page, enhancing data accuracy and participant engagement in the study process. The Oxford WebQ automatically processed the dietary data to calculate nutrient intakes based on UK-specific food composition tables, facilitating the generation of comprehensive nutrient profiles without the need for manual coding by nutritionists. This automated approach ensured efficiency and consistency in dietary data processing, crucial for conducting large-scale epidemiological analyses.

### 4.3. Mediterranean Diet Score (MDS)

Adherence to the Mediterranean Diet (MD) was assessed using the Mediterranean Diet Score (MDS), a validated scoring system based on the method proposed by Trichopoulou et al. (2003). The MDS evaluates adherence to key components of the Mediterranean diet pattern, which is characterized by high consumption of fruits, vegetables, legumes, nuts, whole grains, fish, and olive oil, moderate intake of dairy products, and low consumption of red and processed meats. Each participant received an MDS ranging from 0 to 9, with higher scores indicating greater adherence to the Mediterranean diet.

The calculation of the MDS from Oxford WebQ data involved assigning scores based on predefined thresholds for each dietary component. The score ranged from 0 to 9 and assessed adherence to a Mediterranean diet using intake thresholds for nine components: vegetables, legumes, fruits and nuts, cereals, fish and seafood, monounsaturated to saturated fat ratio, dairy products, meat, and alcohol. Higher scores indicated greater adherence to the Mediterranean diet.

### 4.4. Demographics and Health Information

Demographic characteristics, socioeconomic status, and various health indicators were assessed through structured questionnaires administered during the UK Biobank assessment visits. Key demographic variables included age, sex, ethnicity, and residential location, which were self-reported by participants. Socioeconomic status was evaluated using the Townsend Deprivation Index, a composite measure based on census data that considers factors such as employment, education, housing, and income levels to assess relative deprivation within local neighborhoods.

Health information gathered included participants' medical history, including self-reported conditions such as diabetes, cardiovascular diseases, and other chronic illnesses. Body mass index (BMI) was calculated from measured height and weight data collected during the assessment visits and categorized according to World Health Organization (WHO) standards into normal weight, overweight, and obese categories. Smoking status (current, former, never smoker) and physical activity levels (sedentary, moderate, vigorous) were also assessed through self-report, providing

additional insights into participants' lifestyle behaviors and potential confounding factors in MS risk analyses.

### 4.5. Multiple Sclerosis Events

Occurrences of multiple sclerosis (MS) within the study cohort were identified through linkage to NHS hospital in-patient records, where MS diagnoses were recorded using International Classification of Diseases, Tenth Revision (ICD-10) codes specific to MS (G35). The follow-up duration for each participant was calculated from the date of initial dietary data collection until the first documented MS event, participant death, relocation, withdrawal from the study, or the end of the study period. Participants with multiple MS events contributed data for each occurrence, ensuring comprehensive event ascertainment and follow-up.

### 4.6. Statistical Analysis

Data analyses were conducted using STATA version 18.0 statistical software, employing rigorous analytical methods to explore associations between dietary factors, lifestyle variables, and MS risk within the UK Biobank cohort. Descriptive statistics were used to summarize participant characteristics, dietary intake patterns, and key variables of interest. Cox proportional hazards models were employed to estimate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) to assess the relationship between adherence to the Mediterranean diet (MDS) and MS risk. Models were adjusted for potential confounders, including age, sex, socioeconomic status (Townsend Deprivation Index), smoking status, physical activity levels, and BMI categories. Sensitivity analyses were performed to assess the robustness of findings and ensure consistency across different analytical approaches.

Statistical significance was defined as $p < 0.05$, indicating strong evidence against the null hypothesis and supporting meaningful associations between dietary factors and MS risk. This comprehensive analytical approach aimed to uncover nuanced relationships and potential mechanisms underlying the influence of dietary patterns on MS onset, providing valuable insights for future research and public health interventions aimed at reducing MS incidence and improving outcomes for affected individuals.

# 5. Results

## 5.1. Smoking and Physical inactivity as risk factor for MS

Multiple sclerosis (MS), an autoimmune disease with a higher prevalence in women, showed significant gender-based differences in risk. Men had a notably lower risk of developing MS compared to female participants, with a Hazard Ratio (HR) of 0.59 (95% CI: 0.49-0.72). Age was also a critical factor, with participants aged 51-60 years and those over 60 years having a 31% and 45% lower risk of developing MS, respectively, compared to those aged 50 years and younger.

*Table 1 Distribution of the main characteristics of the participants by event occurrence status and their respective hazard ratios (HR) and 95% confidence intervals (CI) obtained from a univariable Cox regression model*

| | **Multiple Sclerosis (MS)** | | | |
|---|---|---|---|---|
| | **No** | **Yes** | **All** | **HR (95%CI)** |
| | **N=499,085** | **N=478** | **N=499,563** | |
| | **N (%)** | **N (%)** | **N (%)** | |
| **Sex** | | | | |
| Females | 271,124 (54.32) | 321 (67.15) | 271,445 (54.34) | |
| Males | 227,961 (45.68) | 157 (32.85) | 228,118 (45.66) | 0.59 (0.49–0.72) |
| **Age** | | | | |
| ≤50 | 131,053 (26.26) | 178 (37.24) | 131,231 (26.27) | |
| (50-60) | 175,978 (35.26) | 163 (34.10) | 176,141 (35.26) | 0.69 (0.56– 0.85) |
| >60 | 192,053 (38.48) | 137 (28.66) | 192,190 (38.47) | 0.55 (0.44 –0.67) |
| **Smoking** | | | | |
| Never smokers | 199,953 (40.23) | 164 (34.53) | 200,117 (40.23) | |
| Current smokers | 52,576 (10.58) | 80 (16.84) | 52,656 (10.58) | 1.92 (1.47–2.51) |
| Past smokers | 244,469 (49.19) | 231 (48.63) | 244,700(49.19) | 1.16 (0.95–1.42) |
| **BMI (kg/m2)** | | | | |
| Normal (≤25) | 164,075 (33.05) | 160 (34.04) | 164,235 (33.05) | |
| Overweight (26-30) | 210,993 (42.49) | 192 (40.85) | 211,185 (42.49) | 0.94 (0.76–1.15) |
| Obese (>30) | 121,446 (24.46) | 118 (25.11) | 121,564 (24.46) | 1.01 (0.79–1.28) |

*Table 1. Cont.*

| | Multiple Sclerosis (MS) | | | |
|---|---|---|---|---|
| | No | Yes | All | HR (95%CI) |
| | N=499,085 | N=478 | N=499,563 | |
| | N (%) | N (%) | N (%) | |
| **Moderate/vigorous physical activity (days/week)** | | | | |
| 0 | 61,576 (12.62) | 96 (20.87) | 61,672 (12.63) | |
| 1-3 | 181,170 (37.14) | 148 (32.17) | 181,318 (37.13) | 0.52 (0.40–0.67) |
| 4-6 | 153,067 (31.38) | 138 (30.00) | 153,205 (31.37) | 0.57 (0.44–0.74) |
| 7 | 92,045 (18.87) | 78 (16.96) | 92,123 (18.87) | 0.54 (0.40–0.73) |
| **Townsend deprivation index** | | | | |
| Q1 | 124,794 (25.04) | 120 (25.10) | 124,914 (25.04) | |
| Q2 | 124,66 (25.01) | 125 (26.15) | 124,786 (25.01) | 1.05 (0.82–1.34) |
| Q3 | 124,680 (25.01) | 110 (23.01) | 124,790 (25.01) | 0.93 (0.72–1.20) |
| Q4 | 124,332 (24.94) | 123 (25.73) | 124,455 (24.94) | 1.05 (0.82–1.35) |

**Smoking Habits:**

Smoking significantly influenced MS risk in this study. Current smokers demonstrated a 92% higher risk of developing MS compared to never smokers (HR = 1.92, 95% CI: 1.47-2.51). Interestingly, past smoking did not show an increased risk of MS development, indicating a potential reversible effect upon cessation.

**Physical Activity:**

Physical activity levels also played a crucial role in MS risk reduction. Engaging in moderate to vigorous physical activity 1-3 times per week (HR = 0.52, 95% CI: 0.40-0.67), 4-6 times per week (HR = 0.57, 95% CI: 0.44-0.74), or daily (HR = 0.54, 95% CI: 0.40-0.73) was associated with a significant reduction in MS risk compared to no physical activity

**BMI and Socioeconomic Factors:**

Body Mass Index (BMI) did not show a significant association with MS risk in this study, suggesting that weight status may not be a primary risk factor among the study participants.

Similarly, the Townsend Deprivation Index, a measure of socioeconomic status, did not significantly influence MS risk.

These findings provide valuable insights into the influence of smoking habits, physical activity levels, BMI, and socioeconomic factors on MS risk within this study population. Future research should further investigate these factors in larger, more diverse cohorts to confirm these findings and explore underlying mechanisms. Such studies are essential for developing targeted interventions aimed at reducing MS incidence and improving outcomes for at-risk populations.

## 5.2. Oily Fish Consumption and Alcohol Intake in Mitigating MS Risk

**Oily Fish Consumption:**

The study found that consuming oily fish is significantly associated with a reduced risk of developing MS. Participants who consumed oily fish more than twice per week, once a week, and less than once a week demonstrated a 34%, 31%, and 18% lower risk of developing MS at any given time, respectively, compared to those who did not consume oily fish at all. This association was statistically significant, with a p-value of 0.007, indicating robust evidence of its protective effect.

*Table 2 Food distribution and Associations with MS: Hazard Ratios, 95% CI, and P-Values from Univariable and Multivariable Models*

| | Multiple Sclerosis (MS) | | | | |
|---|---|---|---|---|---|
| | **No (Nonevent)** | **Yes (Events)** | **HR (95%CI) unadjusted** | **HR (95%CI) adjusted** | **P value** |
| **Cooked Vegetables (tbs/day)** | | | | | |
| ≤1 | 90,234 | 100 | | | |
| 2 | 163,057 | 153 | 0.84 (0.66–1.09) | 0.95 (0.73-1.24) | |
| ≥3 | 239,138 | 216 | 0.82 (0.64–1.03) | 0.93 (0.72- 1.19) | 0.568 |
| **Sala/Raw Vegetables (tbs/day)** | | | | | |
| ≤1 | 218,611 | 222 | | | |
| 2 | 119,209 | 99 | 0.82 (0.64–1.03) | 0.81 (0.63–1.03) | |
| 3 | 69,846) | 67 | 0.94 (0.71–1.24) | 0.92(0.67–1.23) | |
| ≥4 | 84,505 | 82 | 0.95 (0.74 –1.23) | 0.92 (0.70–1.12) | 0.497 |

*Table 2. Cont.*

| Multiple Sclerosis (MS) | | | | | |
|---|---|---|---|---|---|
| | **No (Nonevent)** | **Yes (Events)** | **HR (95%CI) unadjusted** | **HR (95%CI) adjusted** | **P value** |
| **Fresh Fruit (serving/day)** | | | | | |
| ≤1 | 176,411 | 162 | | | |
| 2 | 140,179 | 136 | 1.05 (0.84–1.32) | 1.15 (0.91–1.5) | |
| 3 | 99,641 | 84 | 0.91 (0.70 –1.18) | 0.94 (0.71–1.24) | |
| ≥4 | 80,523 | 94 | 1.26 (0.98 –1.62) | 1.35 (1.03–1.77) | 0.135 |
| **Oily Fish (times/week)** | | | | | |
| 0 | 54,516 | 76 | | | |
| <1 | 164,055 | 176 | 0.77 (0.59 –1.00) | 0.82 (0.62–1.01) | |
| 1 | 187,599 | 153 | 0.58 (0.44–0.77) | 0.66 (0.49 –0.88) | |
| ≥2 | 89,747 | 70 | 0.56 (0.41–0.78) | 0.69 (0.49 –0.96) | 0.007 |
| **Non-Oily Fish (times/week)** | | | | | |
| <1 | 167,452 | 164 | | | |
| 1 | 247,203 | 226 | 0.93 (0.76 –1.14) | 1.02 (0.83–1.26) | |
| ≥2 | 81,622 | 83 | 1.04 (0.80 –1.35) | 1.09 (0.83–1.43) | 0.568 |
| **Processed meat (times/week)** | | | | | |
| 0 | 46,465 | 48 | | | |
| <1 | 151,533 | 159 | 1.01 (0.73 –1.40) | 1.02 (0.73–1.43) | |
| 1 | 145,317 | 130 | 0.87 (0.62 –1.21) | 0.98 (0.7–1.39) | |
| ≥2 | 154,438 | 140 | 0.89 (0.64–1.23) | 1.03 (0.73–1.46) | 0.936 |
| **Poultry (times/week)** | | | | | |
| <1 | 79,108 | 75 | | | |
| 1 | 178,769 | 172 | 1.01 (0.77 –1.32) | 1.09 (0.82- 1.45) | |
| ≥2 | 240,036 | 231 | 1.01 (0.77 –1.30) | 1.01 (0.77- 1.33) | 0.848 |
| **Beef (times/week)** | | | | | |
| 0 | 55,237 | 59 | | | |
| <1 | 22,5999 | 218 | 0.90 (0.68 –1.20) | 1.01 (0.75–1.37) | |
| 1 | 157,962 | 141 | 0.84 (0.62 –1.13) | 0.96 (0.69–1.32) | |
| ≥2 | 57,475 | 56 | 0.91 (0.63 –1.31) | 1.04 (0.71 –1.54) | 0.982 |
| **Lamb/mutton (times/week)** | | | | | |
| 0 | 88,136 | 86 | | | |
| <1 | 280,355 | 275 | 1.01(0.79 –1.28) | 1.11(0.86–1.43) | |
| ≥1 | 127,022 | 112 | 0.91(0.69 –1.21) | 1.12 (0.83–1.50) | 0.481 |
| **Pork (times/week)** | | | | | |
| 0 | 85,949 | 89 | | | |
| <1 | 281,39 | 261 | 0.90(0.70 –1.14) | 0.98(0.76– 1.26) | |
| ≥1 | 128,358 | 124 | 0.94(0.72 –1.24) | 1.06(0.80– 1.42) | 0.607 |

*Table 2. Cont.*

| | Multiple Sclerosis (MS) | | | | |
|---|---|---|---|---|---|
| | **No (Nonevent)** | **Yes (Events)** | **HR (95%CI) unadjusted** | **HR (95%CI) adjusted** | **P value** |
| **Cheese (times/week)** | | | | | |
| <1 | 97,782 | 96 | | | |
| 1 | 104,303 | 97 | 0.94 (0.71–1.25) | 0.99 (0.75–1.34) | |
| ≥2 | 283,552 | 272 | 0.97 (0.77–1.23) | 1.04 (0.82–1.33) | 0.694 |
| **Bread (slices/week)** | | | | | |
| ≤7 | 157,749 | 182 | | | |
| 8-13 | 196,090 | 177 | 0.78 (0.64– 0.97) | 0.88 (0.71–1.09) | |
| ≥14 | 135,401 | 107 | 0.69(0.54–0.88) | 0.82 (0.63–1.06) | 0.116 |
| **Cereals (bowls/week)** | | | | | |
| ≤3 | 175,617 | 171 | | | |
| 4-6 | 132,729 | 136 | 1.04 (0.83 –1.30) | 1.10 (0.87–1.40) | |
| ≥7 | 188,557 | 168 | 0.91( 0.74 –1.13) | 1.03 (0.82–1.30) | 0.781 |
| **Tea(cups/day)** | | | | | |
| 0 | 73,049 | 77 | | | |
| ≤2 | 129,025 | 116 | 0.85 (0.64–1.13) | 0.88 (0.65–1.18) | |
| 3-4 | 145,820 | 125 | 0.81(0.61–1.07) | 0.92 (0.67–1.23) | |
| ≥5 | 149,903 | 158 | 1.00(0.76–1.31) | 1.07 (0.81–1.41) | 0.376 |
| **Alcohol consumption (drinks/duration)** | | | | | |
| Monthly | 153,520 | 182 | | | |
| Weekly | 243,663 | 216 | 0.74 (0.61–0.90) | 0.78 (0.65 –0.90) | |
| Daily | 101,297 | 80 | 0.67 (0.51–0.87) | 0.73 (0.62–1.02) | 0.854 |

## Alcohol Intake:

Similarly, alcohol consumption emerged as a protective factor against MS onset. Participants who consumed alcohol weekly had a 22% lower risk of developing MS compared to those who drank alcohol monthly or less frequently. This finding underscores the potential protective role of moderate alcohol consumption in mitigating MS risk.

These detailed findings highlight the protective effects of both oily fish consumption and alcohol intake against MS onset within this study cohort.

## 5.3. Mediterranean Diet Adherence and Risk of MS onset

Adherence to the Mediterranean Diet (MD) was examined in relation to the risk of developing multiple sclerosis (MS) in this study. The Mediterranean Diet Score (MDS), which increases by one point per improved adherence level, was used to assess dietary patterns.

*Table 3 Association between adherence to MD and MS risk of onset; calculated using MDS score. Unadjusted and adjusted HRs and corresponding 95% CIs for association with MS.*

| | **HR unadjusted (95%CI)** | **HR adjusted (95%CI)** |
|---|---|---|
| 1-point increase | 0.980 (0.78–1.23) | 0.99 (0.8–1.27) |
| MDS | | |
| <1 | | |
| 1 | 0.68 (0.29–1.57) | 0.71 (0.3–1.64) |
| 2 | 0.62 (0.28–1.40) | 0.69 (0.30–1.56) |
| 3 | 0.80 (0.39–1.63) | 0.90 (0.43–1.84) |

The study found a trend suggesting that higher adherence to the Mediterranean Diet (MD), indicated by an increase in the Mediterranean Diet Score (MDS), and may be associated with a reduced risk of MS onset. However, these associations were not statistically significant in both unadjusted and adjusted models.

Specifically:

- Each one-point increase in MDS showed a slight protective effect against MS onset, although not statistically significant (HR unadjusted = 0.980, 95% CI: 0.78–1.23; HR adjusted = 0.99, 95% CI: 0.8–1.27).

These detailed findings provide preliminary insights into the potential role of Mediterranean Diet adherence in MS risk. While the results did not reach statistical significance, they suggest a trend towards a protective effect with higher adherence to the Mediterranean Diet.

## 6. Discussion

MS is a chronic autoimmune disease whose etiology is considered multifactorial in which the interaction of genetic, immunity and environmental factor plays a role. Among an environmental factor, diet has become one of the components that can be considered for its role in risk of onset of MS. This study has demonstrated that Oily fish consumption and alcohol intake decreasing risk of developing MS than those who don't consume them at all. In addition to diet, Behavioral factors also considered in this study. Among this, Smoking and less physical exercise showed an increase risk in developing MS.

Smoking is a significant environmental risk factor for MS that can be modified. Case-control studies have demonstrated that being a non-smoker reduces the risk of developing MS by at least 13%. There is a complex interplay between smoking and other risk factors for MS, including the HLA-DRB1*15:01 gene, obesity, sun exposure, and Epstein-Barr virus (EBV) seropositivity. (Manouchehrinia, Huang et.al 2022).

Smoking affects the immune system at the cellular level by promoting the development of proinflammatory cytokines, which contribute to sustained autoimmunity. It irritates the lungs, leading to the formation of autoreactive T cells that cross-react with central nervous system (CNS) antigens, triggering an autoimmune response against the CNS and resulting in multiple sclerosis (MS). Research indicates that tissue damage in MS stems from an abnormal immune response to one or more myelin antigens. This response occurs in genetically susceptible individuals after exposure to risk factors such as cigarette smoke. Therefore, quitting smoking could help reduce abnormal immune responses and prevent tissue damage. (Nishanth, Tariq, et.al 2020). Our data clearly show that current smokers have a higher risk of developing multiple sclerosis (MS), whereas former smokers do not exhibit this increased risk. This finding contradicts a meta-analysis, which showed that a history of smoking increases the risk of developing multiple sclerosis (MS) by more than 50% compared to non-smokers. (Zhang, Wang et.al 2016)

A review done by Wingerchuk, 2012, summarized evidence supporting the association between smoking and the risk of developing MS. This review demonstrated that cigarette smoking is an independent risk factor for MS susceptibility, with a relative risk of approximately 1.5. Moreover,

smoking is linked to a higher likelihood of developing progressive MS and experiencing more rapid disability.

Another meta-analysis by Handel et al. (2011) emphasized that smoking plays a significant role in determining susceptibility to MS, although its effect on disease progression remains less certain. The study reported a highly significant increased risk of MS associated with smoking, with no significant statistical heterogeneity observed. It highlighted the importance of considering the dose of smoking and its interaction with susceptibility to MS. The association between the degree of susceptibility and pack-years smoked suggests that smoking may exert a stronger effect on susceptibility earlier in life, but the exact daily cigarette consumption data was not consistently available across studies, posing challenges for meta-analysis.

In our study based on the UK Biobank, we were limited to assessing the smoking status whether they were past smokers, current smokers, or had never smoked. Unfortunately, we were unable to explore detailed information such as the amount (dose) of smoking history or the intensity of smoking and its specific association with the development of MS. This limitation means that while we could analyze overall smoking status in relation to MS risk, we were unable to delve into more nuanced aspects of smoking behavior that could potentially influence MS susceptibility.

The risk of multiple sclerosis (MS) associated with body mass index (BMI) across the entire BMI range remains poorly understood. However, certain studies have provided accurate estimates regarding the age at which obesity is most likely to pose a risk factor for MS. Specifically, according to Hedstrom et al. (2016), the critical period appears to be during adolescence rather than during childhood (the first few years of life). (Hedstrom, Olsson et.al 2016). Mendelian randomization studies have demonstrated that genetic determinants linked to high BMI are associated with an elevated risk of multiple sclerosis (MS). These studies reveal a direct association between increased genetic BMI and MS risk, indicating that obesity plays a causal role in the development of MS. While it has long been established that obesity is a risk factor for various health conditions, particularly cardiovascular diseases later in life, this research underscores the significant implications of obesity during childhood and/or early adulthood for the onset of MS. (Morky, Ross et.al 2016)

A recent Mendelian Randomization (MR) study investigated the potential impact of smoking on the risk of developing multiple sclerosis (MS). The study utilized genetic data to assess whether there was a causal relationship between smoking and MS. The results indicated that there was no significant evidence suggesting that smoking influences the likelihood of developing MS. Specifically, the odds ratio (OR) for smoking initiation was 1.03, with a 95% confidence interval (CI) ranging from 0.92 to 1.61. Similarly, the OR for lifetime smoking was 1.10, with a 95% CI of 0.87 to 1.40. These findings imply that smoking does not have a harmful effect on MS susceptibility. (Mitchell, Bates et al. 2020)

However, it is important to note that these results contradict the conclusions of our study and other recent research. Our study, along with other recent investigations, suggests that smoking might increase the risk of developing MS. The discrepancy between the MR study and other studies highlights the complexity of the relationship between smoking and MS and suggests that further research is needed to fully understand the potential impact of smoking on MS development.

A population-based cohort study in Sweden has showed a higher BMI and obesity are linked to increased levels of neuro-inflammation (such as leptin, interleukin-6, and C-reactive protein) and lower levels of vitamin D. (Xu, Hiyoshi et.al 2021). However, this study demonstrates, indicating no association between BMI and the risk of onset of multiple sclerosis (MS). This lack of association could be attributed to the BMI data from the UK Biobank, used in our study, did not include measurements from adolescence or childhood. Therefore, early-life weight information was not available for analysis.

Several epidemiological studies have indicated a potential link between physical activity and the risk of multiple sclerosis (MS). However, the results have been inconsistent, leaving it unclear whether this connection is causal or influenced by other factors. A Mendelian randomization study has revealed a significant genetic correlation between moderate physical activity and a reduced risk of MS, suggesting that increased levels of moderate exercise are significantly associated with a lower likelihood of developing the disease. (Li, Lin et.al 2022) The Environmental Risk Factors in MS (EnvIMS) study, a multinational case-control research project, demonstrated that vigorous physical activity is inversely associated with the risk of developing MS. (Wesnes, Myhr et. al

2018) This study also aligns with the above findings, suggesting that moderate to vigorous physical activity serves as a protective factor against MS.

Regarding oily fish, this study clearly demonstrated that consuming it more than twice a week has a significantly protective effect against MS. In contrast, eating oily fish less than once a week offers less protection compared to consuming it more than twice a week. Therefore, as the frequency of oily fish intake increases, the risk of developing MS decreases.
Supporting this outcome, a study by Langer-Gould et al. 2020 showed that consuming oily fish at least once a week or at least once a month was associated with a 44% reduced risk of MS onset compared to consuming oily fish less than once a month or not at all. (Langer-Gould, Black et.al 2020)

In addition, The Ausimmune Study, a case-control study conducted between 2003 and 2006, revealed an association between higher fish consumption and a reduced risk of central nervous system (CNS) demyelination. The study found that increasing tinned fish intake by two servings per week was linked to a 40% lower risk of CNS demyelination. Tinned fish, primarily oily fish, is a rich source of vitamin D and polyunsaturated fatty acids (PUFAs). In contrast, grilled fish contains lower levels of both vitamin D and PUFAs, and no association was found between the consumption of grilled or fried fish and the risk of CNS demyelination. (Black, Zhao et.al 2020) This study also supports the current findings from the UK Biobank data.

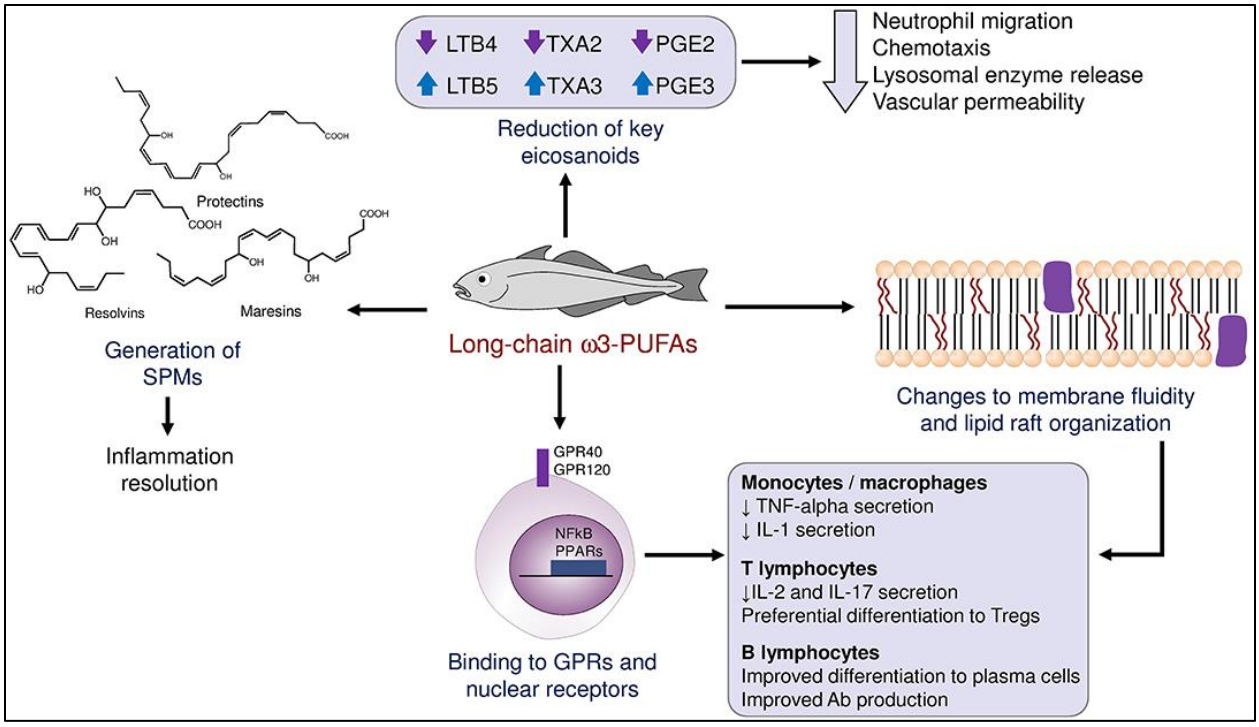


*Figure 5 Mechanisms through which Omega 3-PUFA influence the immune response and local inflammation (Mendivil, 2021)*

Omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are primarily found in fish and other seafood. They possess anti-inflammatory, antithrombotic, and immunomodulatory activities. These fatty acids exert significant effects on gene expression by inhibiting transcription factors like NF-kB, SREBP-1c, and LXR, while activating the nuclear receptor PPAR. Consequently, Omega-3 PUFAs reduce inflammatory processes and fatty acid synthesis, while promoting fatty acid oxidation. DHA is notably concentrated in the brain, though its levels decrease in patients with multiple sclerosis (MS). (Riccio, 2011)

Experimental and clinical studies have shown that Omega-3 PUFAs potentially play a role in preventing the occurrence of neurodegenerative disorders such as Alzheimer's disease, multiple sclerosis, and Parkinson's disease, which are primarily characterized by inflammation.

Omega-3 PUFAs are known to mitigate the onset of the above disorders through their anti-inflammatory and immune-modifying properties. (Parolini 2020) A randomized controlled clinical

trial also demonstrated that MS patients who took Omega-3 PUFAs and Vitamin D supplements together for 12 weeks experienced beneficial effects on their MS disability, inflammation, antioxidant capacity, and metabolic status, including insulin metabolism. (Ebrahim, Mariyam et.al 2018)

The results from our study indicate that frequent alcohol consumption, whether on a daily or weekly basis, is inversely related to the risk of developing MS.

Given that the pathogenesis of multiple sclerosis (MS) is autoimmune-mediated and inflammatory, a recent review focused on the immune system found that acute and moderate alcohol intake weakens the overall state of the immune system and its ability to respond to pathogens. In contrast, chronic alcohol consumption also impairs the general condition of the immune system but results in an increased immune response to pathogens (Fahim, Rafiee Zadeh et al., 2020). Alcohol affects various immune cells and their functions, impacting MS in several ways. It reduces the size and number of lymphocytes in the thymus, spleen, and lymph nodes. Additionally, alcohol impairs the delayed-type hypersensitivity response and diminishes the function of monocytes as antigen-presenting cells.

However, this study did not recommend any specific therapeutic dosages or frequencies of alcohol consumption for immune system suppression due to insufficient evidence. Further research is needed to consider alcohol use as a potential therapeutic factor.

Supporting the results of this study, a meta-analysis of observational studies found no evidence that alcohol consumption is associated with an increased risk of multiple sclerosis (MS). The analysis suggests there may be a potential protective effect of alcohol consumption on the incidence of MS, although this trend is not definitive and requires further validation through additional research. Future prospective cohort studies examining the relationship between alcohol consumption and MS, with a focus on the dosage of alcohol intake, are needed to provide more conclusive insights. (Zu, Ye et.al 2015)

In contrast to this study, a Mendelian randomization study showed that genetically predicted higher alcohol intake was causally associated with an increased risk of MS onset and greater severity of the disease. It also supports that there have been inconsistent findings regarding the associations between alcohol and MS risk, indicating uncertainty that necessitates further in-depth research. This includes distinguishing between the types of alcohol consumed and exploring their relationships with different subtypes of MS. (Nan H, 2024)

Research on the link between alcohol consumption and the risk of developing multiple sclerosis (MS) has shown inconsistent results. A meta-analysis of observational studies found no significant overall association between alcohol intake and MS risk, with an odds ratio (OR) of 0.94, a 95% confidence interval (CI) of 0.73 to 1.22, and a p-value of 0.668. However, a subgroup analysis revealed that beer consumption was linked to an increased risk of MS, with an OR of 1.58, a 95% CI of 1.12 to 2.23, and a p-value of 0.010. (Xu, Qiao et.al 2022) The researchers concluded that beer intake might elevate the risk of MS.

The above findings contradict our study, which indicates that weekly alcohol consumption may protect against the onset of MS, although the specific type of beverage consumed is unknown. Despite this, the study also recommends conducting large-scale prospective studies to confirm these findings. Our study, based on a large cohort from the UK Biobank, offers new insights, even though it does not include information on the type of beverage consumed.

This study however has limitation in terms of the outline of the FFQ administered to participants which did not give an option to detailed analysis of the type of alcohol consumed that are helpful in determination of risk of onset of MS. Thus further studies have to be conducted in order to confirm the association of alcohol and Multiple sclerosis.

In Nutritional Science, diet is a complex variable that requires multiple approaches to examine its relationship with disease risk. Dietary pattern analysis is one such approach, complementing traditional nutrient and food analysis rather than replacing them. This approach is not optimal for effects caused by specific nutrients (e.g., folic acid and neural tube defects) as these effects may be diluted. However, it is useful when traditional nutrient analyses have found few dietary associations with a disease (e.g., breast cancer). Conversely, when many dietary associations exist

(e.g., CHD), dietary pattern analysis can be beneficial as it evaluates the overall diet's impact. Additionally, dietary patterns can serve as covariates in nutrient-specific analyses to determine if a nutrient's effect is independent of the overall diet. (Hu,2002)
This study prospectively evaluated a large cohort of participants from the UK Biobank. Among dietary patterns, the Mediterranean diet has gained interest as a preventive measure for non-infectious diseases.

Several meta-analyses and studies have demonstrated the protective role of adherence to the Mediterranean diet in developing neurodegenerative disorders. (Sofi, Macchi et.al 2013, Gardener and MR Canunca 2018).

The only study so far that explored the link between following a Mediterranean diet and the risk of developing MS was carried out in Iran. This research found that sticking closely to a Mediterranean diet was linked to a lower risk of MS. Specifically, people in the highest adherence group (the third tertile) had a 77% lower risk of MS compared to those in the lowest adherence group (the first tertile), with an adjusted odd ratio (aOR) of 0.23, a 95% confidence interval (CI) of 0.06 to 0.89, and a trend p-value of 0.04. (Sedaghat, Jessri et.al 2016)

A recent comprehensive review examined 29 meta-analyses on the connection between following a Mediterranean diet and 37 different health outcomes, which included neurodegenerative diseases but did not specifically address MS (Dinu, Pagliai et al., 2018). This review supports the idea that higher adherence to a Mediterranean diet lowers the risk of various health issues, such as overall mortality, cardiovascular diseases, diabetes, overall cancer incidence, Alzheimer's disease, and dementia.

The effect of Medritranean diet in Neurroddegeneration and autoimmune diseases can be attributed for its antinflammatory, antioxidant, neuroprotective properties specifically polyphenol for the case of Alzihmer and parkison disease. The evidence of MD in other autoimmne diseases sussch as MS, is SCFASs shown to have immunomodulatory effect and flavinoidds with a remyelination-promoting property. In addition of MD effect on Neurodegeneration disorders and Autoimmune

disease disorder such as multiple sclerosis, it has an immense beneficial effect on multiple system organs in the body. (Gantenbein and Kanaka Gantenbein, 2021)

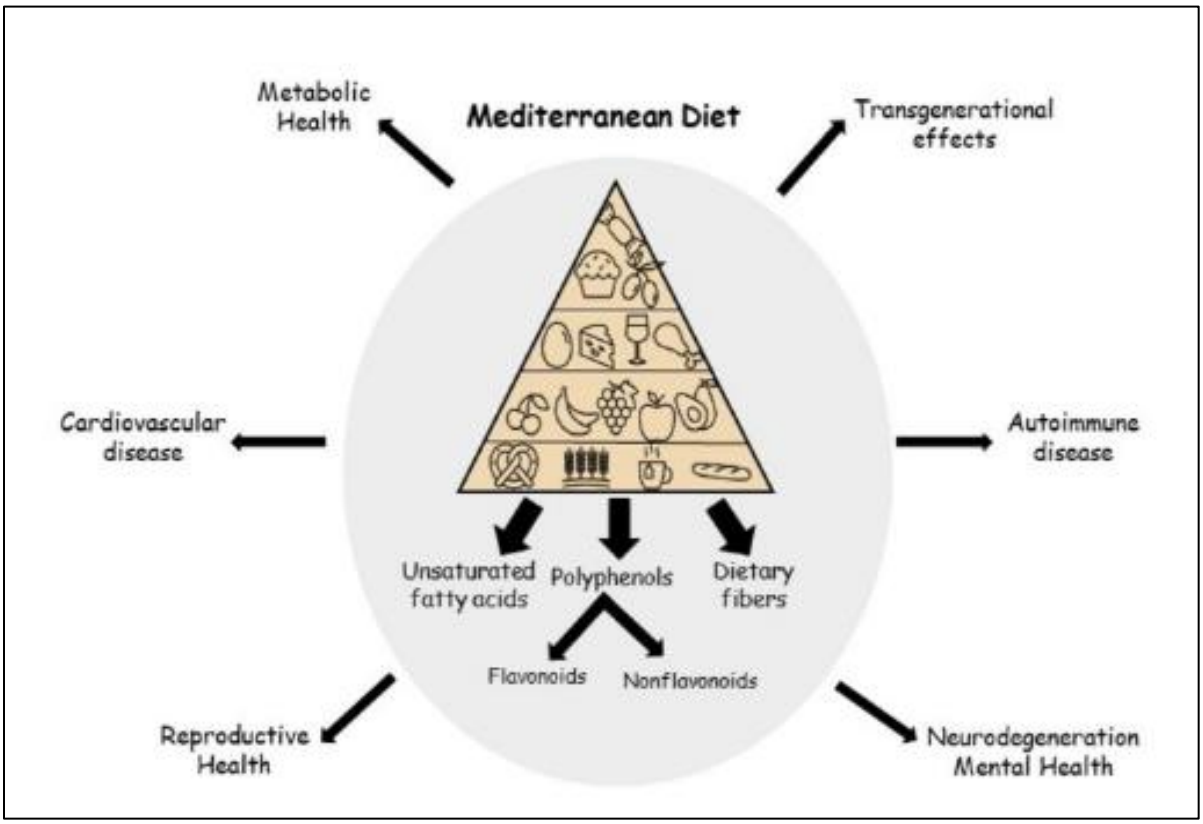


*Figure 6 Beneficial effects of the Mediterranean diet ((Gantenbein and Kanaka Gantenbein, 2021)*

Therefore, it is beneficial to assess adherence to the Mediterranean diet using the Oxford WEBQ, as the FFQ was considered too generic for this specific purpose. MDS system has shown to be specific and sensitive comparing to other studies and highlighted the importance of this score in intervention-based research.

The MDS has been evaluated as useful for assessing individual nutritional status and studying the Mediterranean diet's relationship with health outcomes. ( Panagiotakos, Pitsavos et.al 2006)
MDS calculations for case and control studies showed a non-significant trend where a 1-point increase was associated with an aHR below 1. So far, only case-control studies have indicated that the Mediterranean diet (MD) is protective against multiple sclerosis (MS). (Sedaghat, Jessri et.al

2016) In contrast, this study is based on the larger cohort data available in the UK Biobank, providing a broader population-based perspective.

Supporting this, large population-based prospective cohort studies have shown that higher adherence to the Mediterranean diet is associated with a reduced risk of dementia, a prominent characteristic of Alzheimer's disease, a neurodegenerative condition. (Shannon, Ranson et al. 2023) Available data, mainly from experimental studies, suggest that combining a balanced diet with a healthy lifestyle can improve various clinical parameters and the quality of life for patients with MS. (Guglielmetti, Ferraris et.al)

### 6.1. Limitations and Perspectives

The Oxford WebQ, utilized as a 24-hour dietary assessment tool in this study, has inherent limitations in assessing long-term dietary patterns. While suitable for capturing short-term intake, its reliance on self-reporting may introduce recall bias and inaccuracies. Future studies should consider complementing it with more comprehensive dietary assessments that capture habitual intake over longer periods to better understand the cumulative effects of diet on multiple sclerosis (MS) risk.

The relatively small number of MS cases in the study cohort may limit the statistical power to detect small but potentially meaningful associations between diet and MS risk. This could lead to underestimation of the true effects, highlighting the need for larger sample sizes in future studies to provide more robust conclusions.

The FFQ used in this study did not differentiate between types of alcohol consumption, which may influence MS risk differently. Future iterations of the FFQ should incorporate detailed assessments of alcohol subtypes (e.g., beer, wine, spirits) to elucidate potential differential effects on MS onset risk. Enhancing the FFQ in this manner would enable more nuanced analyses and provide clearer insights into the role of alcohol in MS etiology.

Future research efforts could benefit from incorporating longitudinal dietary assessments using tools like the Oxford WebQ at multiple time points post-enrollment. This approach would facilitate tracking changes in dietary habits over time and their impact on MS risk progression. Longitudinal data collection can enhance our understanding of how dietary modifications influence disease outcomes and inform personalized dietary recommendations for MS prevention and management.

Genetic factors are known to play a significant role in MS susceptibility and response to dietary interventions. Despite the comprehensive nature of the current study, genetic data were not included. Future studies should integrate genetic information to explore gene-diet interactions and identify novel genetic factors that may modulate the effects of diet on MS onset. This integrative approach would provide a more holistic understanding of MS etiology and potentially uncover new therapeutic targets.

These limitations underscore the complexity of studying diet-disease relationships in MS and highlight opportunities for future research. Addressing these limitations through improved dietary assessment tools, larger sample sizes, detailed alcohol subtype analyses, longitudinal data collection, and integration of genetic data will enhance the validity and applicability of findings. Overcoming these challenges will advance our understanding of how diet influences MS risk and pave the way for more effective dietary strategies in MS prevention and management.

## 7. Conclusion

This prospective population-based cohort study utilized UK Biobank data to investigate the intricate relationship between diet and the onset of multiple sclerosis (MS). Participants completed a detailed Food Frequency Questionnaire (FFQ) during assessment visits, enabling a comprehensive analysis of associations between individual foods and MS onset. The study findings suggest that certain dietary factors, such as moderate oily fish intake and weekly alcohol consumption, may confer a protective effect against MS development.

The study employed the Oxford WebQ, a validated 24-hour recall tool, to assess adherence to the Mediterranean Diet (MD). This methodological approach provided a holistic view of participants' overall dietary patterns, capturing broader dietary behaviors beyond individual food components. The use of such comprehensive tools is critical for understanding the cumulative impact of diet on MS onset, potentially guiding preventive strategies and treatment approaches in the future.

The methodological approach utilized in this study holds promise for future research endeavors aimed at preventing MS and developing personalized treatment strategies. By integrating insights from nutritional science into clinical practice, there is potential to tailor dietary recommendations based on disease phenotype, enhancing personalized care approaches for individuals at risk of or living with MS.

While this study provides valuable insights, several limitations should be acknowledged, including potential recall bias inherent in dietary assessment tools and the observational nature of the study design. Future research should aim to address these limitations through larger, prospective studies with longer follow-up periods to further elucidate the complex interplay between diet and MS risk. Additionally, interventions focusing on dietary modifications should be explored to establish causal relationships and guide evidence-based dietary guidelines for MS prevention and management.

In conclusion, this study contributes valuable evidence linking dietary habits, particularly adherence to the Mediterranean Diet, with MS risk. The protective effects observed for oily fish and moderate alcohol consumption underscore the potential role of diet in MS prevention. By leveraging sophisticated dietary assessment tools and population-based data, this research sets a foundation for advancing personalized nutritional strategies in MS management. Ultimately, integrating these insights into clinical practice and public health initiatives holds promise for reducing MS incidence and improving outcomes for affected individuals worldwide.

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