The Relationships between Physical Activity, Fitness, and Serum Biomarkers of Brain Damage and Repair among Individuals with Multiple Sclerosis

by

© Najmeh Sheikhian

A Thesis submitted to

the School of Graduate Studies

in partial fulfillment of the requirements for the degree of

MSc MED (Neuroscience)

Faculty of Medicine

Memorial University of Newfoundland

October 2024

St. John's Newfoundland and Labrador. Canada

Abstract

Background: Multiple sclerosis (MS) is the most common demyelinating, inflammatory and neurodegenerative disease that impacts the central nervous system (CNS) and can cause significant physical and cognitive disability. Physical activity and cardiorespiratory fitness may influence potential biomarkers such as brain-derived neurotrophic factor (BDNF), neurofilament light chain (NfL), and interleukin-6 (IL-6). I investigated whether fitness and self-reported physical activity were related to blood biomarkers of neuroinflammation and repair in individuals with MS.

Methods: Blood samples were obtained from twenty-four patients with Relapsing-Remitting and Progressive MS (18 women and 6 men) before completing a maximal graded exercise test on a recumbent stepper. Participants reported moderate to vigorous physical activity (MVPA) in the previous 24 hours using the adapted International Physical Activity Questionnaire. Serum levels of BDNF, NfL, and IL-6 were analyzed using enzyme-linked immunosorbent assay (ELISA).

Results: I evaluated the relationships between MVPA, fitness (VO_{2peak}), age, weight and biomarkers (BDNF, IL-6, NfL, and BDNF/IL-6 ratio). Results showed that only serum NfL, weight and VO_{2peak} were significantly correlated ($r= -0.435$, $p= 0.034$, and $r= 0.436$, $p= 0.033$), and that the other biomarkers did not correlate with MVPA, fitness, age, or weight ($p > 0.05$).

Conclusion: Higher serum NfL levels, a neuronal damage biomarker, are associated with lower body weight and better physical fitness. Most of the remaining biomarkers show a sensitivity that is not specific enough to differentiate the relationship of fitness to MVPA. This reflects one of the limitations of my study and shall further be elaborated on in the discussion regarding the limitations of my findings.

Keywords: Multiple Sclerosis, moderate-to-vigorous physical activity, brain-derived neurotrophic factor, interleukin-6, neurofilament light chain

Lay Summary

Multiple sclerosis (MS) is a chronic condition with diverse physical and mental symptoms affecting the brain and spinal cord. Recent research investigates the potential of physical activity in mitigating inflammation and promoting brain repair among MS patients. This study involves 24 individuals diagnosed with relapsing-remitting multiple sclerosis (MS), who provided pre-exercise blood samples and documented their recent levels of moderate to vigorous physical activity. Three molecules – interleukin-6 (IL-6), neurofilament light chain (NfL), and brain-derived neurotrophic factor (BDNF) – were quantitatively assessed and their associations examined. Results revealed a connection between higher NfL levels (an axonal damage marker), lower body weight, and being more physically fit (measured as VO_{2peak}). However, the other markers (BDNF, IL-6, and BDNF/IL-6) remained unaffected by physical activity, fitness, age, or body weight. This study suggests a link between elevated NfL levels, lower body weight, and higher fitness level in individuals with MS, indicating that the other markers may lack sensitivity to reflect the impact of physical activity and fitness in this limited patient cohort.

Acknowledgements

I would like to express my deepest gratitude to my supervisor, Professor Michelle Ploughman, for her unwavering support, invaluable guidance, and insightful feedback throughout the research process. Her expertise and encouragement played a pivotal role in shaping this thesis. I am also grateful to the members of my committee, Dr. Craig Moore and Dr.Zhiwei Gao for taking the time to review my thesis and provide valuable feedback. Their expertise and collaborative spirit greatly enriched the research experience.

I extend my appreciation to my dedicated lab mates, who provided a stimulating intellectual environment and shared their knowledge generously. The collaborative atmosphere in Recovery and Performance Lab significantly contributed to the development and execution of this research.

Special thanks goes to my husband, Mojtaba, whose love, understanding, and encouragement sustained me during the challenging phases of this journey. His unwavering support was my anchor, and I am deeply grateful for his presence in my life.

I extend my heartfelt thanks to my family, for their continuous encouragement and belief in my abilities. Their love and support have been a source of strength, and I am truly blessed to have them by my side.

This thesis represents not only my individual efforts but the collective contributions of a supportive network of mentors, colleagues, and loved ones. I am profoundly grateful for the role each of them played in this academic endeavour.

Table of Contents

LIST of TABLES

LIST of FIGURES

LIST of ABBREVIATIONS

AT: Aerobic Training

BDNF: Brain-Derived Neurotrophic Factor

CNS: Central Nervous System

CSF: Cerebrospinal Fluid

DMDs: Disease-Modifying Drugs

EDSS: Expanded Disability Status Scale

ELISA: Enzyme-linked immunosorbent assay

GXT: Graded Exercise Test

IL-6: Interleukin-6

MRI: Magnetic Resonance Imaging

MS: Multiple Sclerosis

MVPA: Moderate to Vigorous Physical Activity

NfL: Neurofilament Light Chain

PMS: Progressive MS

RRMS: Relapsing-Remitting MS

TNF: Tumor necrosis factor

VO_{2max}: Maximum Volume of Oxygen

VO_{2peak}: Peak Oxygen Uptake

Chapter One: Overview and Introduction

1.1 Multiple Sclerosis

1.1.1 Overview of MS

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) wherein the immune system targets the myelin sheath of neurons in the brain and spinal cord (Buzzard et al., 2017; Tafti et al., 2022). The numerous symptoms of the disease are associated with inflammatory lesions in the CNS and axonal demyelination (Haines et al., 2011). Individuals with MS experience a wide range of symptoms, including motor and cognitive impairments. Symptoms such as diminished limb function and sensation, loss of balance and coordination, fatigue, and a decline in cognitive processing and memory significantly reduced the quality of life for affected individuals (Motl et al., 2010). While MS remains incurable, a spectrum of disease management (e.g. disease-modifying therapies, steroids, and symptomatic treatments) is available to effectively manage the disease, alleviate symptoms, slow its progression, and enhance the quality of life for those affected.

Recent research has significantly deepened our understanding of the immunological and genetic elements that contribute to the pathogenesis of MS. The focus of these investigations is, substantially towards the dysregulated immune system in MS; some are directed at reducing the process of inflammation and modulating the immune response, while others seek to delay disease progression. Furthermore, the discussed studies, as highlighted by Tafti et al. (2022) and J. H. Yang et al. (2022), describe molecular and genetic factors that are related to the development of MS, identify new therapeutic targets, and propose possible new ways of treatment that may find implementation in the near future. Development in imaging and discovery of biomarkers, and a better understanding of immunological response in MS, are opening the path for more

individualized and personalized special treatment modalities (Katsavos & Anagnostouli, 2013). Disease-modifying drugs (DMDs), including interferon-beta and monoclonal antibodies like ocrelizumab, have demonstrated efficacy in clinical trials, providing hope for better long-term outcomes for MS patients (Wiendl et al., 2021). A burgeoning body of research, dedicated to unravelling the intricate mechanisms underlying MS and the development of innovative therapeutic interventions (Hauser & Cree, 2020; He et al., 2020; Yang et al., 2022), has made significant contributions to this evolving field, shedding light on various aspects of the disease.

Emerging research has emphasized the importance of a holistic approach to MS management, encompassing pharmaceutical interventions, lifestyle modifications, and rehabilitation (Wills & Probst, 2022). This approach is crucial for addressing the complex nature of MS and its impact on individuals. Pharmaceutical interventions, such as disease-modifying therapies, play a key role in managing the progression of the disease and reducing relapses (Diouf et al., 2023; Kołtuniuk & Chojdak-Łukasiewicz, 2022). Treatment modalities involve oral medications, injections, or infusions and such strategies as the course of the disease changes may require modifications to treatment methods. Although disease-modifying treatments play a crucial role in managing the progression of the disease, they alone are insufficient for enhancing functional recovery (Ploughman et al., 2022). Lifestyle changes such as aerobic exercise, dietary adjustments, and stress management have shown the potential to enhance the well-being of individuals living with MS (Elkhalii-Wilhelm et al., 2022) and can have a positive effect on the immune system, remyelination and inflammatory processes (Barry et al., 2016). Additionally, rehabilitation programs aim to enhance mobility, strength, and independence for individuals with MS (Amatya et al., 2019). In conclusion, while there is no cure for MS at present, ongoing research efforts offer promising prospects for improved treatments and a better quality of life for those affected by this

challenging condition (Hauser & Cree, 2020; He et al., 2020; Tafti et al., 2022; J. H. Yang et al., 2022).

1.1.2 Epidemiology

The most current evidence of the global prevalence found that about 2.8 million people, or 35.9 out of every 100,000, are currently living with the disease. A dramatic increase in the global prevalence of MS has been observed after 2013, which corresponds to persisting distributional disparities. Diagnosed usually at a mean age of 32 years, Walton et al. (2020) pointed out a pooled incidence rate of MS to be at 2.1 per 100,000 person-years across 75 countries. This gene-combined susceptibility generally arises from complex interactions of environmental exposures, sometimes called the 'perfect storm,' which are key contributors to the root cause for disease development (Waubant et al., 2019). Important environmental risk factors detected include exposure to Epstein-Barr Virus (EBV), geographical variation, low levels of Vitamin D, obesity in early life, and smoking. Notably, EBV infection is singled out as an especially strong risk factor (Guan et al., 2019), with building evidence in various research areas pointing to their centrality in light of MS progression (Hedström, 2023; Soldan & Lieberman, 2023). One compelling cohort study of more than 10 million US Army members followed for over two decades found that EBV-infected individuals had a 32-fold higher risk of MS than their uninfected counterparts (Bar-Or et al., 2022; Bjornevik et al., 2022).

From this pattern, the role of environmental factors can be seen to be at the center of the geography of MS. This geographic variation underlines the impact of the environment on MS since MS is found to be highly prevalent in temperate regions and low in Asia and tropical areas. Research has revealed a connection between an increase in the level of disability in patients

suffering from MS and vitamin D deficiency (Alharbi, 2015). There is also a significant relationship between childhood and adolescent obesity or being overweight and susceptibility to developing MS (Loonstra, de Ruiter, Strijbis, et al., 2023). Smoking is also related to an acceleration of the processes described above, from CIS to the confirmed diagnosis of MS and the accumulation of disability in the established forms of the disease (Wingerchuk, 2012).

MS prevalence is higher in certain ethnic communities, women, and family clusters, suggesting a genetic susceptibility (Langer-Gould et al., 2013; Wallin et al., 2012). There have been reports of changes in MS's demographics worldwide during the past few years (Chinea et al., 2017; Lee et al., 2015). A retrospective cohort study based on Kaiser Permanente data in Southern California found that African Americans had a 47% higher risk of MS, in contrast to white Americans, Asian Americans and Hispanic Americans. Hispanic Americans and white Americans had 80% and 50% lower risk, respectively (2.9 per 100,000 for Hispanics vs. 6.9 for whites) (Langer-Gould et al., 2013). Similar findings were reported in a study of the US military Veteran population, indicating higher MS rates in African Americans. Both studies consistently showed lower MS risk in Hispanics and Asians (Wallin et al., 2012). A Canadian study observed a doubling of MS incidence among females of Asian background in British Columbia from 1986 to 2010, unlike the non-Asian white population (Lee et al., 2015).

MS disproportionately affects women, with hormonal factors implicated in this gender disparity (Boziki et al., 2023). The complexity of MS pathways necessitates further investigation into the interplay between sex hormones and genetic factors. Remarkably, there is a higher transmission rate to daughters than sons in families with MS (odds ratios, 2.72 and 1.65, respectively) (Westerlind et al., 2014).

The increasing incidence of MS that tends to cluster in families provides strong support for genetic involvement. The MS concordance rate increases with genetic similarity, with monozygotic twins showing a higher rate than dizygotic twins. However, the lack of full concordance in monozygotic twins and the large differences between monozygotic and dizygotic twins suggest the influence of environmental factors (Sadovnick, 2019). In conclusion, the intricate interplay of genetic, environmental, and lifestyle factors regulates the immune system, contributing to the dysregulation seen in MS. Despite advances in understanding, the precise cause of MS remains largely unknown, emphasizing the need for continued research for effective management.

1.1.3 Pathogenesis

MS is intricately characterized by autoimmune inflammatory processes and neurodegenerative elements, with the disruption of the blood-brain barrier and immune cell infiltration as pivotal hallmarks (Lassmann, 2018). The blood-brain barrier, a regulated protective barrier composed of endothelial cells, typically ensures homeostasis and neural function by selectively controlling the movement of ions, molecules, and immune cells between the blood vessels and the CNS (Daneman & Prat, 2015). In MS, a compromised blood-brain barrier allows the infiltration of leukocytes, including T cells, B cells, and macrophages, into the CNS, contributing to the disease's pathogenesis (Balasa et al., 2021; van Langelaar et al., 2020).

The initiation of MS pathogenesis is debated between two models: the "outside-in" model, suggesting peripheral autoimmune inflammation preceding myelin degradation, and the "inside-out" model, proposing a neurodegenerative event triggering inflammatory responses (Titus et al.,

2020). Regardless of the model, both scenarios lead to an immune response cascade, causing focal inflammation and subsequent destruction of oligodendrocytes—the myelinating cells of the CNS (Stadelmann et al., 2019). The myelin sheath, crucial for efficient nerve conduction, becomes a target for immune-mediated destruction in individuals with MS, resulting in lesions in the brain and spinal cord and contributing to diverse symptomatic manifestations of the disease (Ghasemi et al., 2017). The evolving understanding of MS pathophysiology, including the blood-brain barrier's role and the interplay of inflammatory and neurodegenerative processes, underscores the complexity of the disease and guides ongoing research to develop targeted therapeutic strategies.

1.1.4 Disease Course and Types of MS

The McDonald criteria have been instrumental as a guideline for clinicians, offering guidelines on the earliest diagnosis in the case of MS (Porter et al., 2020; Thompson, Banwell, et al., 2018). These help to work toward the improvement of outcomes and quality of life in individuals who are diagnosed with MS. Recognition of the actual type of MS is fundamental in the standard tailoring of treatment plans and, eventually, guiding research into new therapies that meet individual needs, thereby enhancing the effectiveness of care and treatment (Gajofatto & Benedetti, 2015). There are different types of MS, including relapsing-remitting, primary progressive, and secondary progressive MS.

1.1.4.1 Clinically Isolated Syndrome (CIS)

Clinically isolated syndrome encompasses an initial monophasic episode of clinical symptoms and objective evidence that are suggestive of MS with a duration of at least 24 hours,

along with a lack of fever, infection, and encephalopathy (Grzegorski & Losy, 2020). These episodes are the first demyelinating symptom in ~85% of individuals with MS. The CIS is termed active if a magnetic resonance imaging detects signs of disease activity, such as new or unmistakably expanding T2 lesions on serial imaging (Grzegorski & Losy, 2020).

1.1.4.2 Relapsing-Remitting MS (RRMS)

The prevalent MS phenotype, observed in approximately 85% of individuals with MS, is known as relapsing-remitting MS (Klineova & Lublin, 2018). This type is marked by recurrent episodes of neurological dysfunction, known as relapses, interspersed with periods of relative clinical stability devoid of new neurological symptoms, referred to as remissions (Klineova & Lublin, 2018). While the frequency of relapses may differ among patients, it typically remains below 1.5 per year (Klineova & Lublin, 2018). In the absence of infection or metabolic disturbance, some neurological symptoms, including weakness, altered feeling, impaired balance, decreased visual acuity, and double vision, may be present for at least 24 hours during the relapse (Klineova & Lublin, 2018).

1.1.4.3 Primary Progressive MS (PPMS)

PPMS affects around 10-15% of individuals with MS and is characterized by a slow deterioration of symptoms from the onset of the illness, with no symptom flare-ups or recovery (Braune et al., 2023). While females are more likely to develop relapsing-remitting MS than males, both sexes are equally affected by PPMS (Miller & Leary, 2007). In this type of MS the symptoms worsen gradually and steadily without any relapses or remissions (Miller & Leary, 2007).

1.1.4.4 Secondary Progressive MS (SPMS)

SPMS typically develops in individuals with relapsing-remitting MS after a period of time (usually several years). In secondary progressive MS, the disease gradually progresses with or without relapses or remissions (Ziemssen et al., 2023). The lack of clear clinical, radiological, immunologic, or pathologic criteria that distinguish relapsing-remitting MS from secondary progressive multiple sclerosis, presents a number of difficulties in the accurate diagnosis and clinical management of SPMS (Ziemssen et al., 2023).

1.1.5 Neurodegeneration and Lesion Formation

In MS, both white matter and grey matter are affected by neurodegeneration. The development of focal inflammatory and demyelinating lesions in the white matter regions of the CNS is a hallmark of MS (Lassmann, 2018; Mey et al., 2023). Research has shown that abnormalities in strategic brain white matter tracts contribute to cognitive impairment in MS (Enzinger & Fazekas, 2015). Lesions are present in the grey matter, including the cortex, the grey matter of the spinal cord, basal ganglia, and brain stem, leading to axonal loss in the normal-appearing white matter and diffuse neurodegeneration in the entire grey matter, resulting in profound brain tissue loss and atrophy, particularly in the progressive stage of the disease (Enzinger & Fazekas, 2015; Lassmann, 2018). Furthermore, the relationship between white matter lesions and grey matter atrophy in MS has been investigated, suggesting that grey matter neurodegeneration is mainly secondary to damage in the white matter during early disease stages while becoming more detached and dominated by other, possibly primary neurodegenerative disease mechanisms in PMS (Lie et al., 2022). This highlights the complex interplay between

neurodegeneration and lesion formation in both white and grey matter in MS, emphasizing the need for a comprehensive understanding of these processes to develop effective treatments.

1.1.6 MS Symptoms

MS exhibits a range of diverse symptoms, varying widely among individuals, including fatigue, numbness, loss of balance, stiffness, pain, bladder issues, muscle weakness, coordination difficulties, vision problems, vertigo, and cognitive issues (Ghasemi et al., 2017; Wajda & Sosnoff, 2015). Optic neuritis – optic nerve inflammation – often initiates MS symptoms, presenting as blurred or double vision and eye movement pain (Malik et al., 2014). Prodromal symptoms preceding clinical onset involve disturbances in urinary, gastrointestinal, and anorectal functions, insomnia, fatigue, depression, anxiety, headaches, and various pain types (Disanto et al., 2018).

Emerging evidence suggests a prodromal state before objective MS findings appear, defined as early signs preceding classical disease onset (Makhani & Tremlett, 2021). Clinical features alone are insufficient to identify MS prodrome because symptoms can be common and nonspecific. However, by combining them with biomarkers and demographic characteristics, we can identify people at the greatest risk of developing a clinical event consistent with MS (Makhani & Tremlett, 2021). The typical biomarkers in cerebrospinal fluid and MS serum have also been found at higher rates before the first demyelinating event. Neurofilament light chain (NfL), a marker for axonal damage, has already been proven to be a helpful biomarker in MS, although it is not yet used routinely in the clinical setting (Matute-Blanch et al., 2018).

NfL serves as a valuable biomarker in MS, reflecting axonal damage. Although not yet widely practiced in the clinic, NfL turns out to have a promising avenue as it takes over as the

prognosticating biomarker for relapsing-remitting and progressive MS in the monitoring of both new clinical relapses and treatment response (Kuhle et al., 2019). This marker is integral for quantifying and monitoring neurodegeneration, with optimized assays enabling its measurement in cerebrospinal fluid (CSF) and blood, enhancing its potential for clinical translation (Freedman et al., 2024). In conclusion, the symptoms of MS, often unpredictable and not always correlating with magnetic resonance imaging lesions, highlight the importance of identifying specific biomarkers to increase the specificity for earlier recognition and management of MS (Tremlett et al., 2022).

1.1.7 Approaches to MS Management

The primary treatment approach for individuals with MS involves the use of disease-modifying drugs, recognized as the first-line therapy. These drugs, including ocrelizumab, beta interferons, glatiramer acetate, teriflunomide, and dimethyl fumarate, interact with the immune system to mitigate disease activity. Disease-modifying drug's role is crucial in reducing the frequency of relapses and may slow the long-term progression of disability associated with MS (Vargas & Tyor, 2017; Wingerchuk & Carter, 2014). Within the Canada stats, approximately 19 disease-modifying therapies are available for managing MS. These therapies are administered in various forms, including pills, injections, or infusions, providing individuals with different options for treatment (data & https://mscanada.ca/managing-ms/treatments-for-multiple-sclerosis#toc--disease-modifying-therapies-).

Physical activity has been emphasized as a potential second-line therapy associated with better clinical outcomes among individuals with MS (Motl et al., 2017; Pilutti et al., 2014).

Involvement in physical activity is associated with better health status, cognition, physical functioning, and mobility in individuals with MS (Kohn et al., 2014; Motl, Fernhall, et al., 2011; Motl, Gappmaier, et al., 2011; Motl, Snook, Wynn, et al., 2008). It also reduced fatigue, levels of anxiety, depression and pain (Motl et al., 2009; Motl, Snook, & Schapiro, 2008; Silveira et al., 2021; Stroud & Minahan, 2009; Suh et al., 2010).

Unfortunately, compared to healthy peers, individuals with MS have lower physical fitness and participate in less physical activity (Kinnett-Hopkins et al., 2017; Motl et al., 2005; Motl et al., 2017). They employ power-saving strategies that may include restricting the magnitude of the day's physical activities, all in an attempt to cut down on fatigue. Paradoxically, these can worsen physical deconditioning and further increase fatigue in a vicious circle (Eldadah, 2010; Neal et al., 2020). Both physical activity and fitness have been linked to disability status and disease progression (Ng & Kent-Braun, 1997; Platta et al., 2016; Ranadive et al., 2012; Sandroff et al., 2012). Research demonstrates that maintaining a physically active lifestyle can enhance immune cell function and lower the chance of relapses (Kalb et al., 2020; Motl et al., 2017; Pilutti et al., 2014; Tallner et al., 2012). Individuals with MS who reported vigorous physical activity for more than 3 weeks showed a reduced risk of MS symptoms (Wesnes et al., 2018). Physical activity and exercise are described as a low-cost disease-modifying therapy that may slow down disability progression, lower the relapse rate, stop the growth of pathology seen on magnetic resonance imaging, and improve neurocognitive function (Motl & Pilutti, 2016).

1.2 Physical Activity and Fitness

1.2.1 What is Physical Activity?

The terms “physical activity,” “moderate to vigorous physical activity,” and “exercise” are distinct concepts. However, they are often used interchangeably and inaccurately (Caspersen et al., 1985). The following are the differences between these terminologies:

Physical activity is any physiological motion that increases energy expenditure above resting levels due to contraction of skeletal muscles (Caspersen et al., 1985). Physical activity can be divided into two categories: occupational and leisure-time physical activity. Occupational physical activity refers to activities performed as part of one's work, such as construction work, gardening, or cleaning. Leisure-time physical activity, on the other hand, refers to the activities that are performed outside of work for the purpose of well-being and improving health, such as walking, cycling, or swimming (Bonekamp et al., 2023).

Moderate to vigorous physical activity (MVPA) is a type of physical activity that raises heart and breathing rates. It often consists of activities that raise an individual’s heart rate to between 3 and 6 metabolic equivalents of tasks (METs), which is 50% to 60% higher than a person’s resting heart rate (MacIntosh et al., 2021). The working metabolic rate to resting metabolic rate ratio is known as the MET. One MET is the energy cost of passively sitting still and corresponds to a caloric intake of 1 kcal/kg/h. Activities having a MET of 2 to less than 3, 3 to less than 5, and 5 or greater are categorized as mild, moderate, and vigorously intensive, respectively, for older persons (more than 55 years of age) (Haskell et al., 2007; Nelson et al., 2007). Moderate-intensity activities include brisk walking, dancing, gardening, etc., whereas examples of vigorous-intensity activities include jogging, running, fast cycling, and fast swimming (MacIntosh et al., 2021). Authorities advise that adults should obtain 150 minutes of moderate to vigorous physical

activity each week to achieve a variety of health benefits, such as improved cardiorespiratory fitness, strong bones, weight control, and a decreased risk of developing health conditions like heart disease, cancer, type 2 diabetes etc. (MacIntosh et al., 2021).

Exercise is a subtype of physical activity that is planned, systematic, and repetitive with the goal of enhancing or maintaining one or more physical fitness-related components (Caspersen et al., 1985). The American College of Sports Medicine's recommendations for physical activity divide exercise into categories based on types (such as walking, flexibility, and resistance exercise), intensities (such as vigorous to moderate exercise), frequency (days/week), and duration (minutes). The American College of Sports Medicine suggests resistance, flexibility, and aerobic exercise as kinds of exercise (Williams & Wilkins, 2009).

1.2.2 What Is Fitness?

Fitness refers to attributes related to the ability to perform physical activity (Caspersen et al., 1985). There are several components of fitness, including muscular strength, cardiorespiratory fitness, flexibility, and body composition. The ability of the lungs, blood vessels and heart to provide oxygen to the muscles during physical activity is called cardiorespiratory or aerobic fitness. The ability of muscles to produce force and maintain that force over time is referred to as muscular strength and endurance. The range of motion around a joint is referred to as flexibility. Body composition is the term used to describe the proportions of fat, muscle, bone, and other body tissues (Caspersen et al., 1985).

Aerobic fitness, called cardiorespiratory fitness within clinical contexts, assesses the organism's proficiency in extracting atmospheric oxygen and converting it into cellular energy.

The measurement commonly employed to quantify aerobic fitness is the maximum oxygen consumption ($\dot{V}O_{2max}$), expressed in milliliters per minute per kilogram. Multiple determinants influence aerobic fitness, including gender, age, genetic predisposition, body composition, medical conditions, historical engagement in physical training, pulmonary and cardiac functionality, properties inherent to skeletal muscles and neural factors (Kujala et al., 2019).

1.3 How Do We Measure Physical Activity and Fitness?

Subjective and objective tools are used to measure physical activity and fitness. The subjective and self-reported measures, which consist of tools such as physical activity questionnaires or diaries, have been the most common measurement types; they are practicable, flexible, low cost, and easy to use (Ainsworth et al., 2015; Tudor-Locke & Myers, 2001; Welk, 2002). Objective methods include measures that directly evaluate one or more aspects of physical activity, such as frequency, intensity, time, and type and may be capable of gathering a series of metrics like the number of steps, minutes of exercise, heart rate, etc. (Strath et al., 2013). The most common tools used are wearable sensors, e.g. accelerometers, pedometers, heart rate monitoring, indirect calorimetry and direct observation (Welk, 2002). Accelerometer-based assessment is currently the most widely used objective method for assessing physical activity levels in individuals with MS (Macdonald et al., 2022). When an accelerometer directly assessed physical activity, it was lower in individuals with MS than in sedentary control subjects. The accelerometer also seems more capable than the 7-day recall questionnaire (as a subjective method) of detecting changes in physical activity between relatively inactive groups (Ng & Kent-Braun, 1997).

1.3.1 How Physical Activity and Cardiorespiratory Fitness Impact MS

As mentioned previously (section 1.1.7), regular physical activity has been associated with improved muscle strength and cardiovascular fitness, as well as improved mood and quality of life among individuals with MS. A systematic review by Kjølhede et al. (2012), included 16 studies, revealed that muscular strength improved in lower-limb maximal voluntary contraction (MVC) between 7–21% and 20–50% improvement in lower-limb one-repetition maximum (Langeskov-Christensen et al., 2015) and (Platta et al., 2016) found that peak oxygen consumption and cardiorespiratory fitness improved in response to exercise training. Depressive symptoms also decrease due to exercise training, as shown by (Ensari et al., 2014), (Dalgas et al., 2015) and (Adamson et al., 2015). In a meta-analysis by Motl & Gosney. (2008), their results with 13 studies showed that exercise training improved the quality of life among individuals with MS.

In addition, some studies suggest that regular physical activity may positively affect MS disease progression and symptom management, which may be achieved through improved cardiovascular fitness, enhanced muscle strength, and even better general functional capacity (Heine et al., 2015). All of them can help reduce fatigue, which is the most debilitating symptom among people with MS. Despite the potential beneficial effects of physical activity, individuals with MS are less physically active compared to healthy populations (Motl et al., 2005). A decreased affinity for physical activity can be attributed to factors such as physical barriers (motor impairment, fatigue, pain/sensory symptoms, and heat sensitivity) and environmental barriers (transportation and accessible exercise facilities) (Halabchi et al., 2017).

Several studies support that long-term, regular, moderate-to-vigorous physical activity may provide disease-modifying and neuroprotective effects in individuals with MS. Studies have reported that physical activity decreases the risk of MS, the severity of symptoms and disease

progression (Dalgas et al., 2019; Wesnes et al., 2018). A study by Rooney et al. (2021) (N = 91) demonstrated that in MS participants, increased physical activity was associated with neuromuscular improvement and physical function, highlighting the importance of physical exercise in improving and preserving motor function in individuals with MS. Another study by Klaren et al. (2015) (N = 39) found that moderate to vigorous physical activity was associated with preserved whole brain white and gray matter and deep grey matter structures (thalamus, hippocampus, putamen, caudate, and pallidum) – regions involved in cognitive and motor functions in individuals with MS. However, the relationship between physical activity and MS is complex. Numerous studies advocate that consistent physical exercise can markedly lower the risk of developing MS and slow down the progression of disability.

However, some research has failed to establish a significant connection between the levels of physical activity and the outcomes of MS (Dalgas et al., 2008). The specific effects of physical activity on disease progression and symptom management are still being investigated (Dalgas & Stenager, 2012; Huynh et al., 2022; Kalb et al., 2020). Such complexity itself adds to the possibility that the impact of exercise on MS could be very varied from one patient to another, based on issues of the type and stage of MS, the level and duration of exercise, and individual patient characteristics (Motl, 2021). As with levels of participation in physical activity, individuals with MS have reduced cardiorespiratory fitness compared to their healthy counterparts; an important metric of health status and wellbeing (Canning & Hicks, 2020; Motl & Baird, 2021). In individuals with MS, having decreased level of cardiorespiratory fitness can have several repercussions. A study by Sebastião et al. (2019) (N = 62), investigated the relationship between instrumental activities of daily living (cooking, cleaning, transportation, laundry, and managing finances) and cardiorespiratory fitness in MS patients and found that lower fitness was linked to reduced ability

to complete daily living activities, subsequently limiting one's independence. In addition, in people living with MS, a lower level of cardiorespiratory fitness was associated with a higher risk for secondary diseases, a reduction in functional ability, and an aggravation of disease symptoms (Heine et al., 2016; Madsen et al., 2019). Lastly, studies have demonstrated that in individuals with MS, better physical fitness and more physical activity resulted in preserved brain volumes and improved nervous system integrity (Motl et al., 2015; Prakash et al., 2010).

In conclusion, regular physical activity offers numerous advantages for individuals with MS, such as enhanced cardiorespiratory fitness, increased muscle strength, and an improved quality of life. Although the precise impact of physical activity on disease progression and symptom management is being actively researched, it is widely recognized that regular physical activity is beneficial for those with MS. In addition, the extent in which participation in physical activity or maintaining fitness offers neuroprotective benefits is not fully understood. However, in general, collective findings suggest that boosting cardiorespiratory fitness could improve various MS outcomes, such as disease severity, functional ability, and cognitive function. To monitor these potential disease-modifying effects of physical activity and cardiorespiratory fitness, it is essential to have reliable and accurate objective measures, such as biomarkers.

Biomarkers obtained from magnetic resonance imaging (MRI), cerebrospinal fluid and blood biomarkers may help elucidate inflammation and repair and whether physical activity and cardiorespiratory fitness are neuroprotective in MS (Hamer & Chida, 2009), and (Paul et al., 2019). MRI is used as one of the most important clinical tools for treatment response, disease activity, and disease diagnosis (Paul et al., 2019). On the other hand, molecular markers derived from biological fluids are easy to quantify and can complement MRI and clinical assessment. Clinically relevant and promising blood and cerebrospinal fluid biomarkers are presented below, which are

helpful for the diagnosis and prognosis of multiple sclerosis and for the assessment of treatment response and side effects (Ziemssen et al., 2019).

1.4 Biomarkers/ Cytokines

1.4.1 What Are Biomarkers?

The Food and Drug Administration - National Institutes of Health Biomarker Working Group has defined a biomarker as a defined property that can be measured to reveal pathogenic processes, normal biological processes, or reactions to treatment (Robb et al., 2016). The biomarker concept has changed over the past 50 years to reflect advances in science and medicine. In 1973, the term "biomarker" was first used to denote the existence or absence of a biological material (García-Gutiérrez et al., 2020). Currently, biomarkers can be classified into the following categories: risk/susceptibility, diagnostic, monitoring, pharmacodynamic/response, safety, prognostic, and predictive biomarkers (Figure 1.1) (Cagney et al., 2018) (Figure1.1). An excellent biomarker should be highly reproducible in its measurements, have a high signal-to-noise ratio, and be reliably and dynamically altered when the clinical situation worsens. Additionally, a biomarker should be accessible for detection and measurement, including physiologic, radiographic, histologic, or molecular traits (Robb et al., 2016). Biomarkers can be used to measure and clinically evaluate neurodegenerative diseases such as MS (Ziemssen et al., 2019).

1.4.2 Types of Biomarkers

1.4.2.1 Diagnostic Biomarkers

Diagnostic biomarkers consequently represent very important tools in medicine because they provide information that allows a clinician to confirm or exclude the occurrence of certain diseases or conditions. An example of this is the case of finding certain antibodies in the blood of

a person that will point towards the presence of some infections or autoimmune diseases. This diagnosis is very pivotal in early disease detection and, therefore, accurate disease identification so that proper treatment measures are put in place in good time (Das et al., 2023; Laterza et al., 2007).

1.4.2.2 Monitoring Biomarkers

Monitoring biomarkers are indispensable in the longitudinal observation of a patient's health status. They give much help in tracking, for example, the course of a disease or possibly measuring the effectiveness of a therapeutic effort over time. The biomarkers, whether to measure the effect of the pharmaceutical compound or the effect brought by environmental factors, will provide dynamic insights that help in adjusting treatment plans to bring about better outcomes in patients (Califf, 2018).

1.4.2.3 Pharmacodynamic or Response Biomarkers

Pharmacodynamic or response biomarkers refer to dynamic indicators reflecting changes of the biological pathway or process in relation to the therapeutic intervention. Any alteration of such biomarkers can thus guide clinicians to an informed decision about the continuation or adjustment of treatment. This would, of course, help healthcare providers design treatment regimens for the best results when they understand how a patient's body reacts to a given therapy. This makes therapies more effectual and secure (Okazaki et al., 2018).

1.4.2.4 Predictive Biomarkers

Predictive biomarkers are on the cutting edge of personalized medicine because of their help in finding subjects who are most likely to benefit from the administration of a given medical treatment or intervention. This approach, therefore, optimizes not only the therapeutic effectiveness but also reduces risks of adverse reactions, opening the way toward more targeted and efficient solutions in health care (Cagney et al., 2018).

1.4.2.5 Prognostic Biomarkers

These are commonly used to determine the likelihood that a clinical event may occur in individuals who have been diagnosed with a medical condition or an illness. In clinical trials, prognostic biomarkers are used to identify patients at higher risk of developing clinical events or disease progression. These events can include death, disease progression or recurrence, or the development of a new medical condition. Prognostic biomarkers are also used as inclusion or exclusion criteria in clinical trials to identify populations at higher risk (Carnicer-Cáceres et al., 2021, Califf, 2018; García-Gutiérrez et al., 2020). For example, in the early stages of RRMS, neurofilament light level may serve as a prognostic indicator (Salzer et al., 2010).

1.4.2.6 Safety Biomarkers

Safety biomarkers are essential for evaluating the potential risks associated with medical treatments or environmental exposures. They provide valid information about the likelihood of toxicity and adverse events by giving early signs and indices of harmful effects, which should be monitored. All this approach is proactive in a critical assessment of the safety precautions for patient comfort, decreasing the risk brought about by therapeutic and environmental intervention (Chakrapani, 2020).

1.4.2.7 Susceptibility or Risk Biomarker

This is a risk factor for getting an illness or having a medical condition (Angst & Merikangas, 1997). For the creation of epidemiological studies intended to assess the risk of developing a disease, susceptibility/risk biomarkers are crucial, helping to build preventative methods in clinical practice (Hartwig et al., 2017).

1.4.3 Biomarker Classification in MS

In MS, an ideal biomarker should correlate with disease activity, progression, and treatment effects. MS biomarkers are classified into four categories: 1) disease activity biomarkers can track the activity of the disease and assist in differentiating between various stages of MS progression, such as relapsing-remitting and secondary-progressive MS; 2) predictive biomarkers have the potential to identify individuals who may be at risk of developing MS; 3) diagnostic biomarkers can differentiate MS patients from those with other neurological or autoimmune disorders, as well as from healthy individuals; and 4) treatment-response biomarkers can signify the outcome of a novel treatment in a research context and also monitor the effectiveness of drugs in clinical care (Paul et al., 2019; Ziemssen et al., 2019). Utilizing a combination of imaging biomarkers, such as those derived from magnetic resonance imaging and fluid biomarkers, is vital for clinical MS subtyping, disease monitoring and assessing treatment response (Paul et al., 2019).

1.4.4 Sources of Typical MS Biomarkers

1.4.4.1 Magnetic Resonance Imaging (MRI)

MRI is a non-invasive brain imaging technique that produces three dimensional anatomical images of the body's interior and is often used in clinical practice to detect diseases, confirm diagnoses, and monitor treatments (Hockings et al., 2020; Tomassini & Palace, 2009). In MS, MRI is essential in diagnosis and treatment monitoring as it provides information on the age, size, and number of inflammatory lesions formed in the CNS – including white and grey matter lesions (Comabella et al., 2016; Cook et al., 2012; van Munster & Uitdehaag, 2017). Although imaging biomarkers play a pivotal role in MS clinical care, molecular biomarkers found in the cerebrospinal fluid and blood, comprising of DNA, RNA, and proteins, can complement brain imaging techniques (Ziemssen et al., 2019).

1.4.4.2 Cerebrospinal Fluid (CSF)

CSF is a clear, transparent, protein-rich fluid surrounding the brain and spinal cord and is vital in providing nourishment, waste removal, and protection to the CNS (Telano & Baker, 2018). CSF is mainly produced in the ventricles of the brain by the choroid plexus at a rate of ~500ml/day (Huff et al., 2017). It is an important diagnostic biomarker for a variety of neurodegenerative diseases, such as acute or chronic meningitis, metastasizing tumors, and multiple sclerosis (Péter et al., 2020). A CSF sample can be obtained through an invasive procedure known as a lumbar puncture (spinal tap) in which a needle is inserted into the subarachnoid space of the lumber spine. The collected sample is then tested for abnormalities in red and white blood cells, glucose, and proteins.

In MS, along with MRI identified lesions in the CNS, the analysis of CSF is fundamental in the diagnosis and monitoring of the disease. The presence of oligoclonal bands called immunoglobulins in the CSF indicates chronic immune activation in the CNS and can be detected in several diseases. Oligoclonal bands are created by immunoglobulin antibodies G (IgG) and M (IgM) which are produced by plasma cells in the CNS (Deisenhammer et al., 2019; Ziemssen et al., 2019). Although elevated oligoclonal bands levels is not exclusive to MS, immunoglobulin G (IgG) bands are detectable in the CSF in more than 95% of individuals with MS (Deisenhammer et al., 2019; Ziemssen et al., 2019). Studies have found that oligoclonal bands in the CSF is sensitive and specific for MS and can augment MRI measures to help diagnose and monitor the disease. Therefore, the revised McDonald criteria have accordingly increased the importance of the oligoclonal bands (Deisenhammer et al., 2019; Świderek-Matysiak et al., 2023). Although MRI and CSF analysis are vital diagnostic biomarkers, they lack information on the underlying pathology of MS. Additional biomarkers such as blood biomarkers may be useful to monitor disease activity and detect subtle changes prior to the presence of irreversible MRI detected lesions in the central nervous system.

1.4.4.3 Blood Biomarkers

Blood biomarkers are less invasively collected compared to cerebrospinal fluid and are commonly employed in medicine for treatment monitoring (Ziemssen et al., 2019). These biomarkers allow for repeated sampling and contribute to a shorter diagnostic process, enable efficient monitoring, and streamline the approval of effective treatments for neurodegenerative disorders (Alcolea et al., 2023). Overall, the type of blood marker can be indicative and affiliated with different diseases, such as the link between MS and certain proteins, such as NfL, Brain-

Derived Neurotrophic Factor (BDNF) and Interleukin-6 (IL-6) (Paul et al., 2019; Ziemssen et al., 2019).

1.4.4.3.1 Neurofilament Light Chain (NfL)

Neurofilaments (Nf) are divided into four subunits: α-internexin (Int), neurofilament heavy, medium, and light polypeptides, and NfL (Yan et al., 2007). Nf, and especially NfL, have several traits that make them effective biomarkers of neurodegeneration. NfL can be quantitatively and objectively evaluated. As mentioned, the axonal cytoskeleton's structural scaffolding proteins are called Nf and are necessary for axonal caliber maintenance, stability, radial growth, and electrical impulse transmission (Gnanapavan & Giovannoni, 2015; Yuan et al., 2012; Ziemssen et al., 2019).

It is also particularly sensitive to neurodegenerative processes, and its concentration fluctuates as the disease gets better or worse (Disanto et al., 2017). NfL is a structural cytoskeletal protein that is highly expressed in the axon of neurons. In neurodegenerative diseases such as MS, axonal damage or degeneration results in increased NfL concentrations in the blood and CSF. Although NfL is constantly released from axons in healthy, aging individuals, during axonal damage, NfL concentrations in the CSF can increase to 40 times the baseline level (Ferreira-Atuesta et al., 2021; Gaetani et al., 2019; Ning & Wang, 2022). In MS, several studies have shown that NfL levels are elevated during relapses and reflect MRI -detected lesion development, disease activity, disability, and disease progression (Damasceno et al., 2019; Thebault et al., 2022; Uphaus et al., 2021). It is this disease-specific correlation between elevated NfL levels and MS pathology that distinguishes its diagnostic and prognostic utility from other neurological disorders.

Comparative research supports that the pattern and magnitude of NfL elevations in MS are distinct, correlating with clinical measures of disability and relapse rate, a feature less pronounced in other CNS and PNS diseases. (Ferreira-Atuesta et al., 2021; Ning & Wang, 2022; Ziemssen et al., 2019).

1.4.4.3.2 Brain-Derived Neurotrophic Factor (BDNF)

BDNF is a neurotrophic growth factor expressed in the central and peripheral nervous systems which promotes neuronal differentiation, maturation, axonal growth, and synaptic plasticity (Miranda et al., 2019). BDNF acts by attaching to the tropomyosin receptor kinase B (TrkB) receptor, which in turn stimulates signaling pathways such as MAPK/ERK, PI3K/Akt, and PLCγ to enhance neuronal survival and synaptic strength (Numakawa et al., 2010). Low levels of BDNF have been identified in several CNS diseases including Alzheimer's Disease (Ng et al., 2019) , Parkinson's Disease (Scalzo et al., 2010), Huntington's Disease (Mughal et al., 2011) and MS (Sohrabji et al., 2006). Inflammation triggers immune cells to create neurotrophic factors, such as BDNF, as a coping strategy to protect neurons and promote healing during MS relapses (Caggiula et al., 2005; Sarchielli et al., 2002). Notably, BDNF levels have been observed to rise in the circulation during acute relapses and are upregulated within active inflammatory lesions, suggesting a reactive surge in its production in response to neuronal stress (Azoulay et al., 2008). Moreover, BDNF promotes remyelination by influencing oligodendrocyte precursor cells and enhancing their maturation (Nicholson et al., 2021).

1.4.4.3.3 Pro- and Anti-Inflammatory Cytokines

Cytokines are small secreted proteins involved in the immune response that are responsible for directing cellular communication and are essential for controlling inflammation in the body (Zhang & An, 2007). Various cells generate cytokines, including specific categories such as tumour necrosis factors (TNFs), interleukins (ILs), and chemokines. These molecules play crucial roles in immune cell communication and the regulation of inflammatory responses, and the terminology reflects their diverse roles and sources within the immune system (Becher et al., 2017).

Cytokines interact with receptors on the cell surface, inducing distinct gene expression and mediating intercellular communication. These cytokines have the potential to trigger several cell signaling pathways including autocrine, paracrine, and endocrine signaling (Ramani et al., 2015). Cytokines can have pro- or anti-inflammatory effects in which the balance between the two mediates overall inflammation.

The activity and interaction between pro- and anti-inflammatory cytokines dictate the overall inflammatory response. Pro-inflammatory cytokines are signalling proteins that promote inflammation and are crucial to modulating the immune response. They are produced predominantly by activated macrophages, T cells, and dendritic cells. The pro-inflammatory cytokines interleukin (IL) IL-1 and IL-6, and TNF are involved in up-regulating inflammatory reactions and are associated with the development of pathological pain (Dinarello, 2000; Zhang & An, 2007). When released in response to a threat, pro-inflammatory cytokines signal immune cells to fight invaders and repair tissue damage. While pro-inflammatory cytokines induce inflammation, conversely, anti-inflammatory cytokines suppress inflammation. Major anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13 (Opal

& DePalo, 2000; Zhang & An, 2007). These cytokines collaborate with specific inhibitors and soluble receptors to modulate immune responses. IL-10, for instance, stands out for its potent anti-inflammatory attributes, suppressing the expression of inflammatory cytokines like TNF and IL-1 in activated macrophages. Additionally, IL-6 is a pleiotropic cytokine, meaning it can exhibit both pro-inflammatory and anti-inflammatory properties depending on the context (Opal & DePalo, 2000; Tanaka et al., 2014). Variations in pro- and anti-inflammatory cytokines concentrations in biological fluids such as serum or blood, cannot provide valuable information regarding diagnosis, prognosis and treatment response of various diseases, such as MS (Chow et al., 2022).

1.4.4.3.4 Cytokines in MS

Interleukins are a comprehensive group of cytokines that play a pivotal role in regulating the immune system. They act on processes via cellular adhesion, proliferation, differentiation, maturation, and activation. These cytokines, secreted not only by leukocytes but by a multitude of other cells, have a duality of actions in the immune response: in turn, they act as pro-inflammatory and anti-inflammatory mediators.

The scientific literature extensively documents the involvement of pro-inflammatory and anti-inflammatory cytokines in the context of MS. Pro-inflammatory cytokines, including IL-17, IL-22, TNF, IL-1, IL-12, IL-6 and interferon-γ, have been linked to the promotion of inflammation and tissue damage within the central nervous system, playing a role in the initiation and advancement of MS (Kallaur et al., 2013; Meyer-Arndt et al., 2023; Wang et al., 2018). Conversely, circulating anti-inflammatory cytokines like IL-4 and IL-10 are diminished in MS and are theoretically capable of providing a direct protective effect (Wang et al., 2018). Studies indicate

that pro-inflammatory cytokines are crucial in the pathogenesis of MS, influencing lymphocyte infiltration across the blood-brain barrier and triggering neuroinflammation. In contrast, anti-inflammatory cytokines contribute to a protective role against the progression of the disease (Khaibullin et al., 2017; Vani & Chitra, 2022). The imbalance between pro-inflammatory and anti-inflammatory cytokines is believed to contribute to the development and advancement of MS. Recognizing the distinct roles of various cytokines in the pathogenesis of MS is essential for identifying potential therapeutic targets aimed at modulating cytokine activity and restoring immune balance (Kallaur et al., 2013; Meyer-Arndt et al., 2023; Palle et al., 2017).

1.4.4.3.5 Interleukin-6 (IL-6): A Pro-Inflammatory and Anti- Inflammatory Cytokine

In this manner, the pro-inflammatory action of interleukins, among them, IL-6, is quite important in the first defense reaction to pathogenic agents because they stimulate immune cells and signal them to refer to places of infection or injury. On the other side, their anti-inflammatory effects are of key importance in the resolution process of inflammation to help the inflamed tissue regain its normality before too much damage is reconstituted in the tissue (Vaillant & Qurie, 2022).

IL-6 is a multifunctional prototype cytokine with pivotal importance in the host defense system, as it mediates a broad range of hematopoietic and immune functions (Simpson et al., 1997). Produced by a variety of cell types that include B cells, macrophages, T cells, fibroblasts, and astrocytes, hence its importance in inflammation and immune regulation (Choy & Rose-John, 2017). IL-6 is considered a prominent proinflammatory cytokine that is produced both in response to infectious agents and injury to tissue, with the service of modulating immune responses, including those involved with acute-phase proteins, and also stimulating various cell division and

proliferation events. (Hunter & Jones, 2015) Its proinflammatory effects are generally transduced through a process known as trans-signaling, whereby the soluble IL-6 receptor is engaged, hence allowing activation of STAT3, a pathway normally connected with inflammation and pathogenesis (Garbers et al., 2018).

On the other hand, IL-6 assumes an anti-inflammatory role particularly in the later stages of immune responses. This is achieved through classical signaling, whereby membrane-bound IL-6 receptor is restricted to particular subtypes of immune cells, hence promoting regenerative processes, while in turn, prohibiting the overproduction of pro-inflammatory cytokines (Scheller et al., 2011). In addition, the anti-inflammatory reaction is mediated by IL-6 through stimulation of the production of IL-1ra and IL-10, powerful anti-inflammatory cytokines that usually decrease the inflammatory response (Pedersen & Febbraio, 2008).

1.5 The Relationship between Physical Activity, Fitness, and Blood Biomarkers/Cytokines in MS

1.5.1 Relationship between BDNF, Physical Activity and Cardiorespiratory Fitness in MS

As previously mentioned, BDNF is an essential neurotrophic growth factor in promoting neuroplasticity and has been shown to increase after physical activity. The effect of physical activity and cardiorespiratory fitness on BDNF concentrations is a topic of research interest. (Devasahayam et al., 2021), found that after performing a maximal graded exercise test on a recumbent stepper, BDNF levels were similar in MS and control groups at rest, but interleukin-6 levels were significantly higher in MS. When considering both groups, a higher VO_{2max} was associated with a shift in the BDNF/IL-6 ratio from inflammation to repair. This study provided evidence that enhanced cardiorespiratory fitness in individuals with PMS signifies a shift in the ratio of blood biomarkers toward a repair phenotype.

In the study by Savšek et al. (2021) (N=14), the authors found that BDNF levels increased after 12 weeks aerobic exercise program. In another study (N=94), after 12 weeks (3 sessions per week), the authors demonstrated that exercise training significantly enhanced BDNF levels (Banitalebi et al., 2020). Jeon & Ha. (2017)(N=40) found that the intensity of exercise affected the alterations in BDNF brought on by exercise after 12 weeks of exercise. BDNF increased significantly in the high-intensity exercise group compared to the low-intensity and control group. A meta-analysis review (N= 1,111), showed that frequent exercise, even at a modest intensity, could elevate BDNF levels in individuals with MS, with potential gender differences favoring men over women (Szuhany et al., 2015).

Several studies support that lower intensity and/or shorter duration physical activity had minimal effects on circulating BDNF in individuals with MS. In a study by Abbaspoor et al. (2020) (N=20), they found no significant difference in BDNF levels between a functional training group and the control group after 8 weeks (3 days per week) of rhythmic aerobic exercise, TRX suspension training, elastic band training, and bodyweight training. In another study (N=22), there was no significant difference after 4 weeks of aerobic exercise training (Castellano & White, 2008). However, serum BDNF considerably increased after respondents completed higher intensity ramp tests. These discrepancies may be due to variations in exercise programs' duration, intensity, and type (such as resistance training, aerobic training (AT), or mixed interventions).

Disparate findings in the relationships between physical activity, cardiorespiratory fitness and BDNF in MS could be due to methodological inconsistencies. Timing of blood draws (time of day), handling of the samples, examination of plasma or serum, and the assay used (Bansi et al., 2013) could affect results. Additionally, the differences in findings could be related to the characteristics of the participants, such as gender, MS severity or the type of MS (Mokhtarzade et

al., 2018; Wens et al., 2016), or differences in study design and analysis, such as the sample size and dividing participants into two groups (Ozkul et al., 2018). The measurement of BDNF can be influenced by several factors. Anthropometrics, such as body composition, influences how well BDNF responds to exercise training (Dinoff et al., 2016; Mokhtarzade et al., 2018). Overall, current research supports that 2-3 sessions of moderate-intensity exercise training per week for at least 4 weeks can alter circulating BDNF in people with MS.

There is robust evidence to support the link between higher levels of physical fitness and the upregulation of BDNF. The links between circulating BDNF and neuroprotection have yet to be examined in the context of MS patients, which would require longitudinal monitoring of key biomarkers (Negaresh et al., 2019).

For a more comprehensive understanding of the interaction between physical activity, cardiorespiratory fitness, and their effects on BDNF levels and MS progression, future studies should focus on: establishing causality through longitudinal research (Motl & Pilutti, 2012), uncovering the underlying biological mechanisms (Ploughman et al., 2009), evaluating the role of exercise diversity and intensity (Dalgas, Stenager, & Ingemann-Hansen, 2008), and understanding personal response variations to exercise (Sandroff, Motl, & Scudder, 2016). Additionally, integrating exercise with disease-modifying treatments (Pedersen & Febbraio, 2008), linking BDNF alterations to clinical outcomes (Zimmer et al., 2018), and conducting studies tailored to specific populations (Motl et al., 2017) are essential. There is a critical need for meticulously designed, large-scale studies to investigate the impact of exercise training on MS, particularly from a biomarker perspective (Negaresh et al., 2019).

1.5.2 Relationship between IL-6, Physical Activity and Cardiorespiratory Fitness in MS

IL-6 has emerged as a significant marker in exercise immunology, functioning as a pro-inflammatory and anti-inflammatory agent (Tanaka et al., 2014). In recent years, numerous investigations have explored the physiological benefits of physical activity on the immune system. Physical activity triggers a hormonal pattern that induces an immunologic response (Goldhammer et al., 2005; Steensberg et al., 2001). This response facilitates a balance in the immunologic homeostatic environment by influencing the activity of inflammatory markers (Castellano et al., 2008; Goldhammer et al., 2005; Steensberg et al., 2001) and impacting helper T lymphocytes. It is reasonable to think that along with beneficial effects on physical capabilities, cardiorespiratory function and the individual quality of life, physical activity could have direct influence on immune system function (Castellano et al., 2008; Golzari et al., 2010).

Through physiological processes, physical activity appears to improve functional outcomes and lessen symptoms in MS without exacerbating the inflammatory disease (Florindo, 2014). Numerous cytokines are released into the body during and after physical activity, however it is unclear how these cytokines may affect MS inflammation. Notably, IL-6, an exerkine, exhibits a complex dual-role. It acts as a pro-inflammatory agent when secreted by immune cells during pathological conditions yet adopts an anti-inflammatory profile when produced by muscles in response to exercise. This exercise-induced IL-6 can contribute to the anti-inflammatory environment, potentially mitigating MS-related inflammation. These observations underscore the nuanced influence of physical activity on cytokine profiles and highlight the importance of context in cytokine signalling and its implications for MS (Chow et al., 2022). In a review by Florindo. (2014), aerobic and resistance exercise had a distinct effect on cytokines among persons with MS.

Aerobic exercise increases resistance capacity and overall quality of life, whereas resistance exercise may affect levels of pro-inflammatory cytokines, including TNF- and IFN-gamma, as well as IL-6 and IL-10. These outcomes are associated with the working muscle (Florindo, 2014).

The pro-inflammatory cytokine IL-6, along with being responsive to exercise, is also involved in the pathogenesis of several diseases, including cancer and autoimmune disorders (Scheller et al., 2011). Inflammation depends heavily on pro-inflammatory cytokines like IL-1, TNF, and IL-6 (Hirano, 2021). As mentioned previously, IL-6 can act as both a pro-inflammatory and anti- inflammatory cytokine depending on the context in which it is produced and the cells it is signaling to (Tanaka et al., 2014). When IL-6 is signaling in monocytes or macrophages, it creates a pro-inflammatory response, whereas IL-6 activation and signaling in muscle is anti-inflammatory (Gabay, 2006; Roohi et al., 2021; Tanaka et al., 2014).

Numerous studies have shown that individuals with MS had high levels of IL-6 in their serum and cerebrospinal fluid (Stampanoni Bassi et al., 2020; Stelmasiak et al., 2000). IL-6 can also promote the differentiation of B cells into antibody-secreting cells, leading to the production of autoantibodies against myelin (Sabatino Jr et al., 2019). Whether IL-6 responds to exercise in MS is not clear. Some studies showed no difference in levels of IL-6 after 8 weeks of exercise in individuals with MS. For instance, Schulz et al. (2004) (N=18) found no change after 8 weeks of low-level bicycle exercise training in MS participants with EDSS <5.0. In another study by White et al. (2006) (N=10), IL-6 remained unchanged after 8 weeks of progressive resistance training (twice-weekly). In one systematic review, the authors demonstrated that after regular and acute exercise in individuals with MS compared to controls, IL-6 levels did not show any changes (Shobeiri, Seyedmirzaei, et al., 2022). Additionally, some studies reported no significant changes in IL-6 levels among MS participants after various exercise modalities (e.g., ergometer, aerobic,

combined, resistance exercise) (Alvarenga-Filho et al., 2016; Bansi et al., 2013; Berkowitz et al., 2019; Briken et al., 2016; Deckx et al., 2016; Majdinasab et al., 2018; Schulz et al., 2004; White et al., 2006)

Others found reductions in IL-6 levels following combined training (Faramarzi et al., 2020; Tadayon Zadeh et al., 2021) and 12 weeks of resistance exercise (Kierkegaard et al., 2016). Devasahayam et al. (2021), showed that among persons with progressive MS, as well as age and sex matched controls, being fitter shifted the exercise-induced concentrations of BDNF to IL-6 (increased BDNF and lowered IL-6). Tadayon Zadeh et al. (2021)(N=30) (EDSS≤ 6) found that, after 8 weeks combined exercise training program, IL-6 levels decreased significantly. Castellano et al. (2008) (N=22) showed that the cytokine responses to 8 weeks aerobic exercise in MS patients and the control group varied and it was discovered that both groups' IL-6 levels tended to drop as they trained. Another study by Faramarzi et al. (2020) (N=94) explored the impact of combined exercise training on pentraxins and pro-inflammatory cytokines in individuals with MS after 12 weeks, demonstrating a significant reduction in IL-6 levels following combined exercise training.

In contrast, some studies reported an increase in IL-6 levels after 8 weeks of ergometer use (Barry et al., 2019) and a single session of fitness (Devasahayam et al., 2021). According to Briken et al. (2016) (N=42), patients with PMS exhibited a larger elevation (36.2%) in serum IL-6 levels after graded exercise test (GXT) following 9 weeks of endurance training than a wait-list control group (10.3% increase) ($p = 0.06$). A comprehensive meta-analysis, comprising 226 studies and encompassing 13,526 individuals diagnosed with MS, revealed a noteworthy elevation in the concentrations of 21 blood cytokines, including IL-6, among MS patients compared to control groups (Bai et al., 2019). Additionally, a positive correlation has been found between NfL and both disease activity and progression in MS (J. Yang et al., 2022). These studies support that short

durations of exercise seem to increase IL-6, which is likely released from muscle and anti-inflammatory, while long-term adherence to exercise and higher fitness seems to suppress IL-6 which is likely pro-inflammatory.

In contrast to the variety of trends seen in the MS population, studies show that IL-6 and physical activity are consistently positively correlated in the healthy population. Physical activity appears to have a similar effect on the inflammatory indicators of IL-6 in MS and healthy individuals. The amount of IL-6 released during skeletal muscle activity rose dramatically, which probably had an impact on the synaptic activity, oxidative stress, and inflammation process (Petersen & Pedersen, 2005; Steensberg et al., 2001). The large rise in IL-6 levels may encourage the development of its soluble receptor (sIL-6r) which, when different from Th2, induces the proliferation of tumor necrosis factor, and controls cellular activity in the blood-brain barrier (Neurath & Finotto, 2011). When healthy people engaged in rigorous and prolonged physical exercise (such as high-performance athletes and marathon runners), the results were identical. The tendency of sIL-6r to increase during physical activity in MS exactly matches the outcomes of healthy people following an extended activity (Robson-Ansley et al., 2009; Schulz et al., 2004).

Starkie et al. (2003) (N=8), demonstrated that exercise caused to release IL-6 from the skeletal muscle, which is thought to suppress tumor necrosis factor, a key mediator of inflammation, in healthy volunteers. IL-6 produced during exercise is crucial for preserving homeostasis (Knudsen et al., 2017) and mediating some of the systemic advantages of the exercise (Brown et al., 2015; Pedersen, 2007).

Regarding exercise, its role in modulating inflammation presents a promising avenue for both individuals with MS and healthy populations. It is recognized that exercise has a systemic anti-inflammatory impact, which is crucial for managing chronic conditions like MS, where

inflammation plays a pivotal role in disease progression. However, the specific impact of exercise on IL-6 levels, a key marker of inflammation, in MS patients is nuanced and influenced by several exercise-related factors. Studies have revealed that aerobic exercise can lead to reductions in IL-6 levels in MS patients, suggesting an anti-inflammatory benefit. For instance, (White & Castellano, 2008) demonstrated that regular aerobic exercise could decrease serum IL-6 concentrations, potentially mitigating the inflammatory processes associated with MS progression. Similarly, a systematic review by (Dalgas & Stenager, 2012) highlighted the variable effects of different exercise modalities on IL-6 levels, underscoring the importance of exercise intensity, type, and frequency in eliciting beneficial inflammatory responses.

Moreover, the frequency of exercise sessions plays a critical role in modulating IL-6 levels. Regular, moderate-intensity exercise has been associated with long-term anti-inflammatory effects, as evidenced by lower IL-6 levels in individuals adhering to consistent exercise routines (Motl et al., 2017). In contrast, high-intensity exercise may produce transient increases in IL-6, reflecting an acute inflammatory response that could yield long-term anti-inflammatory benefits through a complex physiological adaptation process (Heine et al., 2015). These findings suggest that while exercise generally promotes an anti-inflammatory state beneficial for MS patients and healthy individuals, the specific outcomes on IL-6 levels depend on the exercise regimen's characteristics. Therefore, tailoring exercise programs to individual needs and capabilities emerges as a critical consideration in leveraging physical activity as a therapeutic strategy for managing MS-related inflammation. In conclusion, IL-6 is an essential inflammatory marker in the pathogenesis of MS, and its levels are elevated in individuals with MS. IL-6 plays a very crucial role in helping control the immune reactions, inflammation, and the healing of tissues. Approaches

targeting IL-6 through the use of monoclonal antibodies and small molecule inhibitors have demonstrated potential in the management of MS.

Current literature identifies several major research gaps that must be studied in more detail to expand our knowledge of the interaction between physical activity, IL-6 modulation, and MS progression. Firstly, the literature presents a paradoxical narrative on the impact of various exercise regimens on IL-6 levels among MS patients, thus suggesting an urgent need for a deeper mechanistic understanding (Gabay, 2006; Roohi et al., 2021; Tanaka et al., 2014). Although the benefits of physical activity are known, the optimal intensity, type, duration, and frequency of exercise that beneficially modulate IL-6 levels and, by extension, exerts influence on MS progression and symptomatology are not adequately defined (Faramarzi et al., 2020; Florindo, 2014; Kierkegaard et al., 2016; Tadayon Zadeh et al., 2021). Secondly, the lack of comprehensive longitudinal investigations precludes a good understanding of the long-term effects of physical activity on IL-6 dynamics and MS disease trajectory (Schulz et al., 2004; White et al., 2006). Thirdly, a great lack of the spectrum of individual variability in response to exercise intervention, apparently influenced by other confounding factors like age, sex, disease severity, and baseline fitness levels, has highlighted a requirement to make tailor-made exercise prescriptions for maximal therapeutic benefits against MS (Petersen & Pedersen, 2005; Neurath & Finotto, 2011). Lastly, there is an evident lack of studies linking immunological alterations with clinical outcomes in MS patients and hence points to a critical gap. Such a linkage will be fundamental in developing a comprehensive understanding of how exercise may serve as a control mechanism in the management of MS (Bai et al., 2019; J. Yang et al., 2022). Addressing these identified gaps would considerably improve our understanding of the intricate relationship between exercise, IL-6

modulation, and MS. This advancement is pivotal for devising more effective, evidence-based physical activity interventions as therapeutic modalities in the comprehensive management of MS.

In summary, IL-6 is a pro-inflammatory and anti-inflammatory actor of exercise immunology. From this point of view, the actor has a capability of exercise immunology to moderate the immune response by turning it down in MS. Physical activity could increase IL-6 production, although its effects on inflammation and progression of MS could be variable, and studies have documented mixed results. The variability underlines the complexity of the IL-6 role and its action by exercise, the personalized regimens in exercise to exploit the therapeutic potentials optimally. Future research would be supposed to capture the exact mechanisms through which the modulation of IL-6 is mediated by exercises and the possible management implications in MS.

1.5.3 Relationship between NfL, Physical Activity and Cardiorespiratory Fitness in MS

A cohort study (N=1158) using data from the Chicago Health and Aging Project found that engaging in physical activity is linked to reduced cognitive decline in older adults who have elevated levels of serum NfL (Desai et al., 2022). In a range of neurological conditions, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases, NfL levels rise in cerebrospinal fluid and blood proportionately to the severity of axonal damage (Gaetani et al., 2019). Recent studies have shown that NfL levels can be used as a neuroaxonal damage biomarker for a variety of neurological conditions, including MS (Gaetani et al., 2019; Kuhle et al., 2019), Alzheimer's disease, and traumatic brain injury (Thebault, Booth, et al., 2020). NfL has been studied as a potential marker for tracking injury and recovery in individuals with neurological conditions. Recent research indicates that serum NfL may be helpful in monitoring treatment response to disease-modifying medication in MS patients (Delcoigne et al., 2020; Disanto et al.,

2017; Kuhle et al., 2019; Novakova et al., 2017). According to numerous studies, NfL levels rise during MS relapses and are correlated with the emergence of MRI lesions (Barro et al., 2018; Disanto et al., 2016; Novakova et al., 2017), disease activity, disability, and disease progression (Thebault, Abdoli, et al., 2020). The patient can safely be measured for NfL, and NfL levels are rather simple to find. New technologies enable quick, easy, and less intrusive measurement techniques. This enables easier sample gathering and storage as well as periodic assessments. NfL has been used as an outcome measure in various clinical trials, and results have demonstrated that disease modifying therapies significantly lower NfL levels when compared to placebo (Axelsson et al., 2014; Gunnarsson et al., 2011; Kuhle et al., 2015; Romme Christensen et al., 2019). NfL is now a useful outcome metric in clinical studies as a result of this discovery (Axelsson et al., 2014).

In the case of people who already have MS, some authors have reported elevated NfL as the only indicator of disease activity in people with PMS vs. Relapsing-Remitting MS (Reyes et al., 2020), while others have discovered greater levels of NfL in Relapsing-Remitting MS vs. PMS (Martin et al., 2019), and that NfL levels at the time of diagnosis correlated with long-term progression from Relapsing-Remitting MS to PMS (Bhan et al., 2018). Several methods, such as ELISA, single-molecule array tests, and electrochemiluminescence immunoassays, can be used to evaluate NfL levels in cerebrospinal fluid or blood samples. These methods rely on antibodies that bind to NfL specifically and enable its measurement (Kuhle et al., 2016).

Recent studies in aging and Huntington's disease have explored the relationships between physical activity and NfL. For instance, in a large sample of older individuals assessed longitudinally (N=1158) among those having lower NfL, engaging in medium and high levels of physical activity was associated with a slower rate of cognitive deterioration compared to those with higher NfL levels (Desai et al., 2022). (Cruickshank et al., 2020), studied the relationships

between serum NfL and lifestyle factors (physical activity, cardiorespiratory fitness, smoking, alcohol use, and extent of social networks) in 29 Huntington's Disease mutation carriers and 15 matched controls. Although there were no differences in NfL between groups, fitness was associated with lower NfL in healthy controls but not in Huntington's patients. Because the use of NfL as a biomarker is a relatively new field, it will take time to determine its relationships to fitness and physical activity in cross-sectional and longitudinal cohorts.

In the field of MS, there is controversy regarding whether NfL is responsive to exercise. For instance, Gravesteijn and group recently reported serum NfL results of a subset of MS patients (30 exercise and 25 no exercise) participating in a randomized controlled trial testing whether 16 weeks of aerobic exercise improved fatigue (Gravesteijn et al., 2023). There were no between group differences in the change in NfL (measured using SiMoa) however the relationships between fitness (an indicator of longer-term adherence to exercise) was not assessed. In another secondary analysis of a trial comparing 3 weeks of high or moderate intensity aerobic exercise among relapsing-remitting and PMS participants, a single acute bout of exercise resulted in immediate reduction in NfL (measured using SiMoa) which returned to baseline 3 hours later. However, there were no differences in NfL between groups after the 3 weeks intervention (Joisten et al., 2021). Several other groups present positive effects of exercise on NfL. For example, (Ercan et al., 2021), examined serum NfL in relapsing-remitting MS patients before and after either 8 weeks of aerobic exercise or a home exercise control condition. They reported that NfL levels (measured using enzyme-linked immunoassay) were significantly lower after 8 weeks only among those participants receiving aerobic exercise. Similarly, in a pilot study among 11 MS patients, 6 weeks of resistance exercise resulted in lowering of plasma NfL (measured using SiMoa) (Ercan et al., 2021; Joisten et al., 2021; Mulero et al., 2023). At this time there are no studies that examine the

extent to which NfL is related to habitual levels of physical activity or to cardiorespiratory fitness in MS patients. There are also no longitudinal studies linking cardiorespiratory fitness to MS progression or to NfL levels. These are critical areas of study in order to determine whether engaging in exercise is neuroprotective in MS.

Overall, the use of NfL as a biomarker in exercise trials in MS shows promise however there are some inconsistencies in how NfL is measured (serum or plasma, type of assay). A recently registered clinical trial (NCT05229861) comparing moderate and high intensity aerobic exercise among people with PMS (Kupjetz et al., 2023) is positioned to evaluate both the effects of exercise on NfL as well as the relationships between baseline fitness and baseline NfL. This will be the first exercise study in MS that pre-specifies using NfL as a biomarker.

In summary, NfL has emerged as an indicator in the field of neurological disorders, including MS. These proteins that provide support play a role in maintaining the size of nerve fibres, their stability and the transmission of electrical signals. Elevated levels of NfL in both CSF and blood have been associated with neurological conditions like MS, Alzheimers disease and traumatic brain injury. Importantly NfL has shown potential as a marker for monitoring disease activity, disability progression and response to treatment in individuals with MS. While there are some debates about whether NfL levels are affected by exercise in MS patients or not. This emphasizes the need for research to establish the connection between physical activity, cardiovascular fitness and NfL levels. The potential use of NfL as an indicator in exercise-based trials for MS holds promise; however, it is crucial to standardize measurement methods. Ongoing clinical trials are expected to provide insights into how exercise affects NfL levels and its correlation with fitness levels. This will contribute to an understanding of exercise's role in managing MS symptoms.

1.6 Relationships between BDNF, IL-6 and NfL and other Lifestyle Factors

It is important to consider that, besides how samples are handled, circulating biomarkers could be influenced by characteristics of the individual such as anthropometrics, age, and smoking or even time-of day (Zielinski & Gibbons, 2022). These factors would likely have to be considered when analyzing biomarker data.

1.6.1 Weight

Several studies confirm there is a link between cytokines and obesity. In general, most cytokines in the interleukin family increase with greater central obesity. Research indicates a pivotal role played by elevated obesity and body mass index in the onset of MS and higher levels of chemerin, an adipokine associated with obesity, have been identified in MS patients with excess weight compared to their non-obese counterparts (Correale & Marrodan, 2022; Schreiner & Genes, 2021). This suggests a plausible interplay among weight, inflammation, and cytokine levels within the context of MS.

Although decreased brain levels of BDNF has been implicated in obesity, particularly in animal models, a large systematic review and meta-analysis confirmed that there were no differences in circulating BDNF in serum or plasma between humans subjects with obesity and controls (Sandrini et al., 2018). One study that examined the role of BDNF in predicting stroke recovery noted as association between body weight and serum BDNF and therefore reported BDNF concentration as a ratio of body weight in kilograms (King et al., 2019). In terms of the associations between NfL and body weight, higher NfL has been shown to be related to lower body weight (and blood volume) (Benkert et al., 2022; Khalil et al., 2020). In a large general population cohort, higher NfL was associated with lower body mass index, Waist-to Hip size Ratio

and less total body water (Hermesdorf et al., 2022). Characteristics of participants in terms of weight, Body Mass Index (BMI) and examination of anthropometrics among sample outliers should be included in studies that examine links between physical activity, fitness and biomarkers (IL-6, BDNF and NfL). When possible, body weight should be controlled for in the analysis.

1.6.2 Age

Aging is described as a gradual decline in the function of different organ systems over time (Ponnappan & Ponnappan, 2011). With advancing age, there is a progressive unfolding array of degenerative processes within the body that work adversely to lower immune competence. This decline in immune competence is described as immunosenescence and involves degradation, attempts to compensate, and rebuild the immune system. Such changes render the elderly much more vulnerable to the entire array of health problems, which include infectious diseases, neoplastic conditions (tumours), and chronic illnesses (Ponnappan & Ponnappan, 2011; Yu & Zheng, 2019). The immune system is a key sentinel for detecting deteriorations that manifest in aging because of its ability to sense and protect against pathogenic signals (Franceschi et al., 2007). The irregular regulation of cytokines is thought to be a crucial factor in the restructuring of the immune system during the aging process. Evidence suggests that the inability to control systemic inflammation precisely is a sign of aging that is not progressing successfully. This transformation in the pattern of cytokine expression, characterized by a gradual shift toward a pro-inflammatory phenotype, is referred to as "inflamm-aging" (Rea et al., 2018).

1.6.2.1 Age and IL-6

Interleukin-6 has been widely acknowledged for its significance in the aging process and age-related illnesses and has been called "the cytokine of gerontologists" (Ershler, 1993; Ershler

& Keller, 2000). In typical circumstances, the blood contains low concentrations of Interleukin-6; however, its levels rise with aging and in individuals exhibiting indicators of frailty and chronic conditions. In such cases, there is a correlation between elevated Interleukin-6 levels and mortality (Puzianowska-Kuźnicka et al., 2016; Van Epps et al., 2016; Varadhan et al., 2014).

1.6.2.2 Age and BDNF

It appears that the regulation of BDNF during the aging process varies depending on the species and the area. It has been reported that BDNF levels rise in the murine hippocampal tissue during normal aging, but not in pathologically altered mice (Katoh-Semba et al., 1998). The intensity of BDNF-immunoreactivity in the cell bodies and dendrites of the neurons in the hippocampus formation has been observed to decrease in aged monkeys (26, 30, and 32 years) (Hayashi et al., 2001). Human age has been observed to correlate with a decline in BDNF levels in plasma (Lommatzsch et al., 2005).

1.6.2.3 Age and NfL

NfLs are one of the most promising neurological blood-based indicators, and they are cytoskeletal proteins that are only expressed in neurons (Ladang et al., 2022). Studies report an increase in NfL levels with increasing age. In a cohort study, after a mean follow-up time of 5.9 years and using a single molecule array (n = 335), they demonstrated that subclinical comorbid diseases might be the cause of the acceleration of neuronal injury at older ages, as indicated by rising and more varied NfL in those over 60 (Khalil et al., 2020). Given that comorbidities are more common in the aged population, it may emerge with age and contribute to the rise in blood NfL levels rather than the aging process alone (Divo et al., 2014).

Understanding the dynamics of NfL is essential in applying blood NfL levels to clinical practice. The precise duration for blood NfL concentrations to peak after neuronal injury and its half-life in the bloodstream remains uncertain. This highlights the significance of obtaining ongoing measurements in each patient over time. The time required to reach the peak of NfL concentrations in the blood following neuronal injury and its half-life in the blood has yet to be precisely discovered. This further underscore the importance of obtaining longitudinal measurements in an individual patient (Barro et al., 2020).

Age-related mechanisms significantly influence the progression of MS. Notably, research has demonstrated a correlation between aging and the transitioning from the relapsing-remitting phase to the progressive phase of MS, typically manifesting in the fifth decade of life (Zeydan & Kantarci, 2020). Furthermore, the age at disease onset has been identified as a key determinant, with older age associated with shorter times to disability levels, suggesting a substantial impact of age on disease progression (Scalfari et al., 2011). While clinical and subclinical disease activity may decrease with aging, the post-relapse recovery potential also decreases with age, and the efficacy of disease-modifying treatments declines with older age (Zeydan & Kantarci, 2020). In summary, age-related factors play a pivotal role in shaping the trajectory of MS and influencing the disease.

1.7 Thesis Objective

The goal of this thesis was to study the relationships between MVPA, cardiorespiratory fitness (VO_{2peak}), and the serum biomarkers BDNF, IL-6 and NfL in individuals with MS. More specifically, we aimed to investigate the physiological processes that underlie the effects of

physical activity and to determine the feasibility of using serum biomarkers BDNF, IL-6, and NfL to monitor the impact of physical activity and cardiorespiratory fitness in individuals with MS based on their fitness level which is measured as a VO_{2peak} and their self-report physical activity. We also considered the relationships between our key biomarkers and potentially important confounders, age, and weight. In the present study, we conducted a cross-sectional study of an MS cohort in which we measured levels of cardiorespiratory fitness (VO_{2peak}) and physical activity as well as serum BDNF, IL-6 and NfL concentrations. We hypothesized that higher levels of MVPA and cardiorespiratory fitness are associated with lower levels of IL-6 and NfL, and higher levels of BDNF in individuals with MS. As physical activity and cardiorespiratory fitness become increasingly important in MS neurorehabilitation, it is vital to continue to investigate non-invasive biomarkers as indicators of treatment-induced effects.

1.8 Coauthor Statement

I conducted this research under the supervision of Professor Michelle Ploughman. Blood, cardiorespiratory fitness, and demographic data were previously collected by laboratory staff. Dr. Ploughman identified the research topic and I designed the research approach. I assisted Dr. Ploughman's' staff in extracting, cleaning and checking participant data. I assisted staff in Dr. Craig Moore's laboratory to complete the assays. I created the spreadsheets and completed the data analysis. I prepared the manuscript with support from Dr. Ploughman, her staff and other laboratory trainees. The manuscript has not been submitted for publication and is formatted in general thesis style.

2 Chapter Two: Manuscript

2.1 Introduction

Multiple Sclerosis (MS) is an autoimmune disease, diagnosed in over 2.5 million people globally, that affects the spinal cord, optic nerves, and brain (Thompson, Baranzini, et al., 2018). Recent research suggests that lifestyle factors can either provide neuroprotection or hasten MS progression. For instance, having cardiovascular comorbidity (Marrie et al., 2010) or smoking (Briggs et al., 2017) is linked to more rapid loss of walking among individuals with MS (Marrie et al., 2010). A growing body of research in animal models and persons living with MS suggests that participating in physical activity can have positive effects on the immune system (Jensen et al., 2018), integrity of the CNS (Chaves et al., 2019; Prakash et al., 2010), and relapse rate (Lozinski & Yong, 2022; Ploughman et al., 2022). However, in the past, persons with MS were advised against physical exercise to avoid exercise-induced increases in body temperature, which worsened feelings of fatigue (Lozinski & Yong, 2022).

New DMDs help thwart relapses, but axonal degeneration and demyelination occur over time, even in the absence of relapse events (Tur et al., 2023). However, a recent systematic review (Sokratous et al., 2023) concluded that evidence was too weak to conclude that cardiorespiratory fitness or physical activity interacts with MS progression. In light of these findings, further research is needed to better understand the complex relationship between lifestyle factors, physical activity, and the course of MS. Such insights could pave the way for more tailored and effective interventions to improve the lives of individuals living with this challenging condition.

Circulating biomarkers such as BDNF, IL-6, and NfL are gaining attention for their roles in indicating the effects of physical activity on individuals with MS, where fitness levels may

influence the shift from pro-inflammatory states towards repair and neuroprotection (Devasahayam et al., 2021; Papathanassoglou et al., 2015), Despite the potential indicated by lower serum matrix metalloproteinases in response to high-intensity interval training (Zimmer et al., 2018) the impact of exercise on other biomarkers like BDNF remains inconclusive. Furthermore, while NfL is emerging as a promising marker for neuronal damage (Bittner et al., 2021), its response to physical activity and its potential to serve as a surrogate marker for MS progression requires further investigation (Gravesteijn et al., 2023; Kupjetz et al., 2023). Exercise also offers benefits for brain health, potentially preventing structural decline (Prakash et al., 2010) and enhancing neuroplasticity by upregulating various neurotrophins (Geng & Mark, 2015; Knaepen et al., 2010; Papathanassoglou et al., 2015). Nonetheless, the diversity in the effects of exercise on CNS inflammation highlights the need for more comprehensive research (Negaresh et al., 2019; Gravesteijn et al., 2023).

Serum biomarkers seem to be responsive to acute bouts of exercise in individuals with MS however, little is known about their relationships with overall physical activity and fitness (Florindo, 2014). Physical activity can be measured either by using accelerometry or by asking people to report their physical activity patterns using standardized questionnaires such as the International Physical Activity Questionnaire (Craig et al., 2003). Aerobic fitness represents the body's ability to extract oxygen to produce energy and is typically measured using the gold standard graded maximal exercise test to derive the maximum volume of oxygen (VO_{2max}) (Bayles, 2023). Engaging in higher levels of physical activity usually leads to improved fitness.

Physical activity is further stratified by intensity using the concept of the metabolic equivalent of a task or MET. Resting MET is 1.0 while moderate to vigorous physical activity would range from 3 to 8 METs (Jetté et al., 1990). MET-based prescription would reflect a wide

range of homeostatic disruptions given that VO_{2max} can range from 5 METs to >20 METs depending on age, sex, genetic predisposition, and individual fitness level (Iannetta et al., 2021). According to Warburton et al. (2011), the prescription for moderate and vigorous exercise intensity may also be based on heart rate ranges expressed relative to an individual's maximal heart rate or percentage of heart rate reserve. Moderate exercise is defined as 40–59% of the aerobic capacity reserve or heart rate reserve, and vigorous exercise is defined as 60–84% of these reserves.

The majority of the MS population does not engage in physical activity of sufficient intensity and volume (Warburton et al., 2007; Borgundvaag and Janssen, 2017) to achieve these health benefits, despite the fact that moderate to vigorous intensity physical activity (MVPA) is frequently recommended for health benefits (Tremblay et al., 2010). We undertook this study to determine the relationships between potentially important blood biomarkers of neuroinflammation (NfL, IL-6) and repair (BDNF and BDNF/IL-6 ratio) and physical activity (MVPA) and cardiorespiratory fitness (VO_{2peak}). We also considered whether serum biomarkers could also be related to relevant demographic and anthropometric variables (age and weight (kg)). We hypothesized that higher levels of MVPA and cardiorespiratory fitness are associated with lower levels of IL-6 and NfL and higher levels of BDNF in individuals with MS.

2.2 Material and Methods

2.2.1 Study Design

We conducted a cross-sectional study of an MS cohort recruited from a specialized MS clinic. Following informed written consent, participants agreed to attend a 3-hour study session in which they first completed demographic information, had anthropometrics measured, and reported

details of physical activity in the previous 24 hours. Just before completing a graded maximal exercise test, blood was drawn from the median cubital vein. The Human Research Ethics Board approved this study (HREB Ref: 2016.1208).

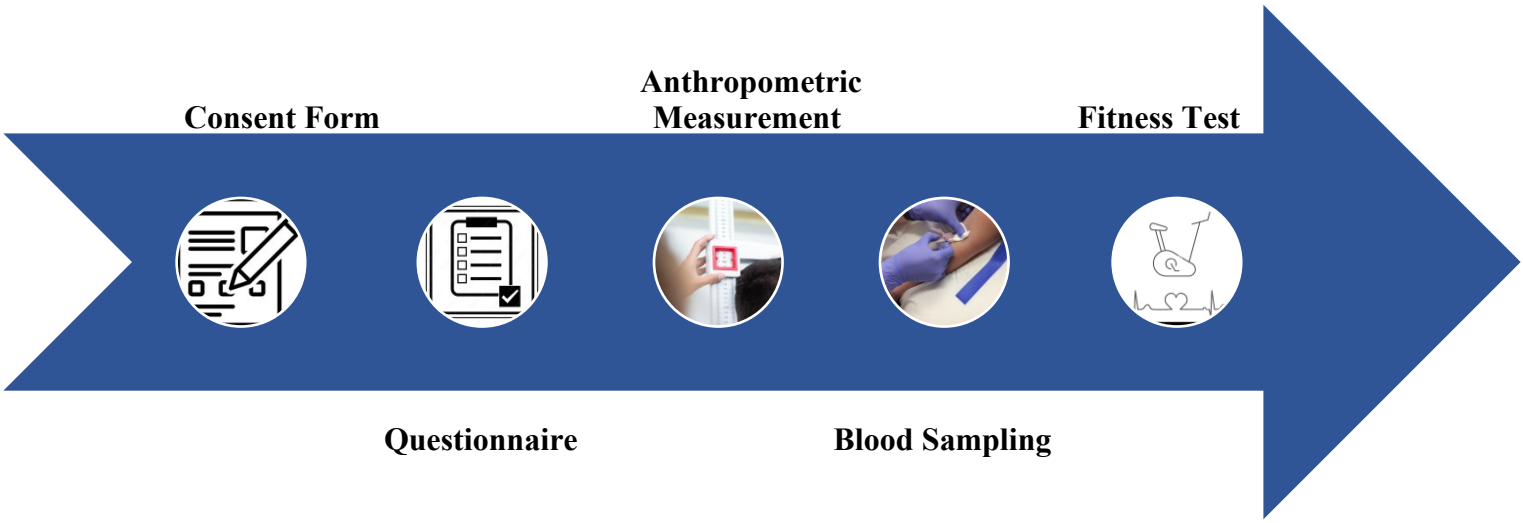

Figure 2.1. Study design

Illustrating the flow of assessments conducted throughout the study, providing a visual overview of the study's experimental timeline (Original Illustration by Najmeh Sheikhian).

2.2.2 Sample Size Calculation

With the alpha set at 5% and a power of 80%, the minimum sample size was estimated to be between 20 and 25 to detect the effects of exercise-induced serum BDNF, NfL and IL-6 (Briken et al., 2016; Gold et al., 2003; Shahim et al., 2016).

2.2.3 Participants

We aimed to recruit at least 20 participants. Participants were included if they: (1) were ≥18 years old, and (2) received a MS diagnosis using the Revised McDonald Criteria (Thompson, Banwell, et al., 2018). They were excluded if they (1) experienced recent active disease (previous 3 months), or (2) were deemed unable to participate in a graded exercise test as per the physical activity readiness questionnaire (PAR-Q) (Shephard, 1988; Thomas et al., 1992). Those participants who failed PAR-Q were referred to a physician for a Physical Activity Readiness Medical Examination (PARmed-X) (Warburton et al., 2011).

2.3 Demographics, Activity and Fitness Outcomes

2.3.1 Demographics

In order to determine the degree of MS-related disability, we extracted the neurologist-scored expanded disability status scale from the participant's health record (Kurtzke, 1983). We also extracted disease duration (years) and MS disease type (relapsing-remitting or progressive). In addition, we measured their body weight using a digital scale (Healthometer Pro+Plus, Healthometer Professional Scales; McCook, Illinois).

2.3.2 Determination of MVPA

We used the adapted International Physical Activity Questionnaire and encouraged participants to list all activities in which they participated during the previous 24 hours, then

calculated that into the metabolic equivalent of task (MET); we consider only MET ≥ 3.0, in the moderate to vigorous range (Medicine, 2013; Piercy et al., 2018). MET-minutes is then computed by multiplying an activity's MET score by the number of minutes the activity was performed. Finally, the resulting MET values were added together to provide a continuous measure of physical activity in the total ME -minutes/week (Motl, Arnett, et al., 2008).

2.3.3 Cardiorespiratory Fitness Test

Before proceeding to the Graded Exercise Test, the participants were instructed to fast for at least four hours. At the beginning, test procedures were fully explained to the participants, they became familiarized with the device, and any questions or doubts they had been resolved. Then the ergometer arm and leg were adjusted based on their height and comfort, after which the participants were equipped with Polar chest sensor for monitoring their heart rate (HR) (H10, Polar Electro Inc., NY, USA). A face mask with a two way non-rebreathing valve was connected by tubing to a metabolic cart (Moxus Metabolic Systems, AEI Technologies, Inc., Pittsburgh, Pennsylvania, USA) that measures the amount of oxygen inhaled and exhaled breath-by-breath, as well as respiratory rate (RR) (Kelly et al., 2017). Blood pressure (BP) was recorded with an automated blood pressure monitor (Physio®logic, A.M.G. Medical Inc., Montreal, QC, Canada).

Exercise began at a load level of 3 (corresponding to 20 W) with a recumbent stepper (NuStep, Ann Arbor, MI, USA) on a standard scale of 1–10 and increased by 20 W every 2 minutes (min). If participants did not stop by load level 10, we increased the stepping rate by 10/min, every 2 min.

Criteria for test termination were:

(1) Volitional exhaustion

(2) Inability to maintain workload

(3) Signs of excessive fatigue

(4) Despite an increase in effort, there was no VO_2 or HR rise (Chaves et al., 2019).

We recorded the volume of oxygen uptake normalized to body mass ($\dot{V}O_{2Peak}$; mL/kg/min), HR (bpm), and rating of perceived exertion (RPE; 10-points) every 2 min during exercise.

Before each test, the gas analyzers were calibrated using standard gases that contained 16.0% oxygen and 4.0 +/- 0.02% carbon dioxide and ambient air (20.94% oxygen and 0.03% carbon dioxide). During graded exercise test (GXT), participants were instructed to keep up a pace of 80 steps per minute while the workload increased by 20 W every two minutes starting at load level 3 (21 W) and continuing until fatigue was reached (Kelly et al., 2017).

The achievement of VO_{2max} during exercise suggests that a person has achieved their genuine physiological limit. A plateau in VO_2 (an increase of less than 150ml/min, despite an increase in workload) is the gold standard for determining if a person has reached their VO_{2max} (Bayles, 2023). Participants in clinical populations rarely reach a VO_2 plateau, despite exerting themselves to the fullest. In clinical populations, VO_{2peak} may be a more representative way to describe fitness levels (Arena et al., 2007). We used the term VO_{2peak} as all the participants were not able to achieve a plateau of VO_2 during the maximal effort due to muscular fatigue, or fatigue unrelated to their cardiorespiratory system. Similar to (Motl & Baird, 2021), VO_{2peak} (ml/kg/min) was determined as the highest recorded 15 second VO_2 value, when at least one of the following

conditions were met: (1) respiratory exchange ratio > 1.10, (2) > 90% age predicted maximal HR, or (3) ≥ 8.0 rate of perceived exertion (ACoS, 2013).

Figure 2.2. Recumbent stepper

(NuStep, Ann Arbor, MI, USA) for measuring $VO_{2peak.}$ Original Photo: Najmeh Sheikhian.

2.3.4 Blood Samples and Processing

Prior to the Graded Exercise Test, blood samples were taken from the median cubital vein and placed in 5mL serum containers (Price et al., 2007). The blood samples were allowed to coagulate for 30 to 60 minutes, then the serum was extracted and stored frozen at -80 °C. According to the manufacturer's instructions, serum levels of BDNF, IL-6, and NfL were tested using ELISA sets for human BDNF (R&D Systems Inc. Minneapolis, MN, USA), IL-6 (BD Biosciences, San Diego, CA, USA) and NfL (Uman Diagnostics, Umeå, Sweden). NfL and IL-6

tests were run in duplicate, and BDNF was in triplicate. The minimum detectable concentrations of BDNF, NfL and IL-6 in serum were determined to be >23.4 pg/mL, >0.4 pg/mL, and >4.7 pg/mL, respectively. We completed the BDNF assay in two dilutions: 1:500 and 1:1000. In preliminary correlation analysis there was no difference between 1:500 and 1:1000, so we proceeded with subsequent analysis with 1:500. A commercially available ELISA (Uman Diagnostics, Umeå, Sweden and BD Biosciences, San Diego, CA, USA, respectively) was used to measure NfL and IL-6 in accordance with the manufacturer's recommendations. To define the shift in the balance of neurotrophic and pro-inflammatory cytokines towards a repair phenotype in MS, we estimated a ratio of BDNF and circulating inflammatory cytokine IL-6 (BDNF/IL-6). Higher VO_{2max} following the Graded Exercise Test was shown to be closely related with a shift in the BDNF/IL-6 ratio from inflammation to repair (Devasahayam et al., 2021).

2.3.5 Statistical Analysis

The assumptions of normality for all variables were verified using histograms, box plots, and Shapiro-Wilk tests ($p > 0.01$) (Ghasemi & Zahediasl, 2012; Öztuna et al., 2006), all conducted within the IBM SPSS statistical software package version 24 (IBM Corporation, NY, USA). Data analysis, including the determination of relationships between serum biomarkers, age, weight, MVPA, and cardiorespiratory fitness, was performed using Spearman's rank correlation coefficient within the same statistical software.

2.4 Results

2.4.1 Participant Characteristics

A total of 25 people with Relapsing-Remitting and Progressive MS (19 women and 6 men) were recruited, but we excluded one because they did not reach VO_{2peak} based on the criterion. The remaining 24 participants were on average 50.5 years of age and were diagnosed with MS for 17.5 years (Table 1).

Table 2.1. Participant characteristics

	Characteristic	Mean(±SD)
Demographic		
Age (years)		50.46(±8.55)
Sex	Female/Male	18/ 6
Weight (kg)		77.84(± 17.44)
Years since MS diagnosis		17.5(±7.7)
Types of MS(n)	RRMS	21
	SPMS	3
EDSS score		2.29(±1.97)
	Dimethyl fumarate	5
	Glatiramer acetate	4
Disease Modifying Therapies	Fingolimod	3
	Teriflunomide	2
	Interferon beta-1a (Avonex)	2

	Interferon beta-1a (Rebif)	1
	None	7
	Smoker/Previous smoker	15
Smoking	Nonsmoker	9
Biomarkers		
BDNF ($pg.ml^{-1}$)		86.03(±12.87)
IL-6 ($pg.ml^{-1}$)		2.56(±1.22)
NfL ($pg.ml^{-1}$)		12.03(± 6.35)
BDNF/IL-6 ratio ($pg.ml^{-1}$)		18.04(± 9.34)
Functional measures		
VO_{2peak} (mL/ min/ kg)		30.12(±8.9)
MVPA in 24 hrs (MET- day/min)		451.70(± 348.45)

kg: kilogram; RRMS: Relapsing remitting MS; SPMS: secondary-progressive MS; EDSS: Expanded Disability Status Scale; BDNF: brain-derived neurotrophic factor; pg: picograms; mL: milli-liter; IL-6: interleukin-6; NfL: Neurofilament Light chain; VO_{2Peak}: peak aerobic capacity; MVPA: moderate to vigorous physical activity; min: minute.

2.4.2 Relationships between Blood Biomarkers, Age, and Weight

Higher serum NfL was significantly correlated with lower body weight (r= -0.435, p= 0.034: Figure 1 (d)). BDNF showed a weak positive, nonsignificant correlation with age (r = 0.209, p = 0.327; (Table 2; Figure 1)), which can be indicative of the tendency of BDNF values to increase

with age. A similar weak and non-significant correlation was noticed for weight (r = 0.125, p = 0.560), where no clear relationship of body weight with BDNF was revealed in our sample.

IL-6 correlated with age and was not associated with age (r = -0.071, p = 0.743)The correlation between IL-6 and weight showed a positive trend (r = 0.244, p = 0.250), suggesting that with an increase in body weight, a proportionate increase in the levels of IL-6 was observed, but this, too, was not statistically significant.

The BDNF/IL-6 ratio was weakly positively correlated with age (r = 0.199, p = 0.351) and weakly negatively correlated with weight (r = −0.206, p = 0.334). These again suggest potential trends but are not significant in our sample. Overall, the serum NfL was significantly correlated only with a low body weight, while the other biomarkers, namely IL-6 and BDNF, showed non-significant trends, underlining their complexity of interaction in terms of age and weight in people with MS. The observed trends in this study, though without statistical significance, are important for understanding the larger framework of biomarker dynamics and should be evaluated in a larger study with better design to gain insights into their potential clinical relevance.

Table 2.2. Blood biomarkers and Spearman's correlation with age and weight

Biomarkers	Age (Years)		Weight (kg)	
	R	P	R	P
BDNF	0.209	0.327	0.125	0.560
IL-6	-0.071	0.743	0.244	0.250
NfL	0.161	0.452	-0.435*	0.034

BDNF/IL-6 ratio	0.199	0.351	-0.206	0.334

kg: kilogram; BDNF: brain-derived neurotrophic factor; IL-6: interleukin-6; NfL: Neurofilament Light chain

2.4.3 Relationships between Blood Biomarkers, MVPA and Fitness

Higher serum NfL was related to higher cardiorespiratory fitness as measured by VO_{2peak} (r= 0.436, p= 0.033: Figure 2 (f)). The other relationships were not significant (p >0.05) (Table 3; Figure 2). For BDNF, there were weak positive, but non-significant, correlations with MVPA and fitness (r = 0.115, p = 0.593), meaning that there were no clear relationships with BDNF levels in our sample.

In terms of IL-6, the correlation with MVPA was positive (r = 0.331, p = 0.114), suggesting a trend towards higher levels of IL-6 with increased physical activity, though nonsignificant. There was no correlation between IL-6 and fitness (r = 0.053, p = 0.806), indicating no significant relationship between IL-6 levels and cardiorespiratory fitness.

Although the BDNF/IL-6 ratio showed a weak negative correlation with MVPA (r = −0.174, p = 0.406) and a weak negative correlation with fitness (r = −0.183, p = 0.391), the findings were not statistically significant in our sample. In summary, the relationship between serum NfL and higher cardiorespiratory fitness was significant. The nonsignificant trends for IL-6 and BDNF thus underline the complexity of their interactions with physical activity and fitness in MS patients. These nonsignificant trends—in the absence of statistical significance—might be of importance for the understanding of the general context of the dynamics of biomarkers and should be further explored with larger sample sizes in more rigorous study designs to tease out the potential clinical implications.

Table 2.3. Blood biomarkers and Spearman's correlation with MVPA and fitness

Biomarkers	MVPA (MET/min)		Fitness (VO_{2peak})	
	R	P	R	P
BDNF	0.105	0.626	0.115	0.593
IL-6	0.331	0.114	0.053	0.806
NfL	-0.012	0.957	0.436**	0.033
BDNF/IL-6 ratio	-0.174	0.406	-0.183	0.391

kg: kilogram; BDNF: brain-derived neurotrophic factor; IL-6: interleukin-6; NfL: Neurofilament Light chain; MVPA: moderate to vigorous physical activity; MET: metabolic equivalent of task; min: minute; VO_{2Peak}: peak aerobic capacity.

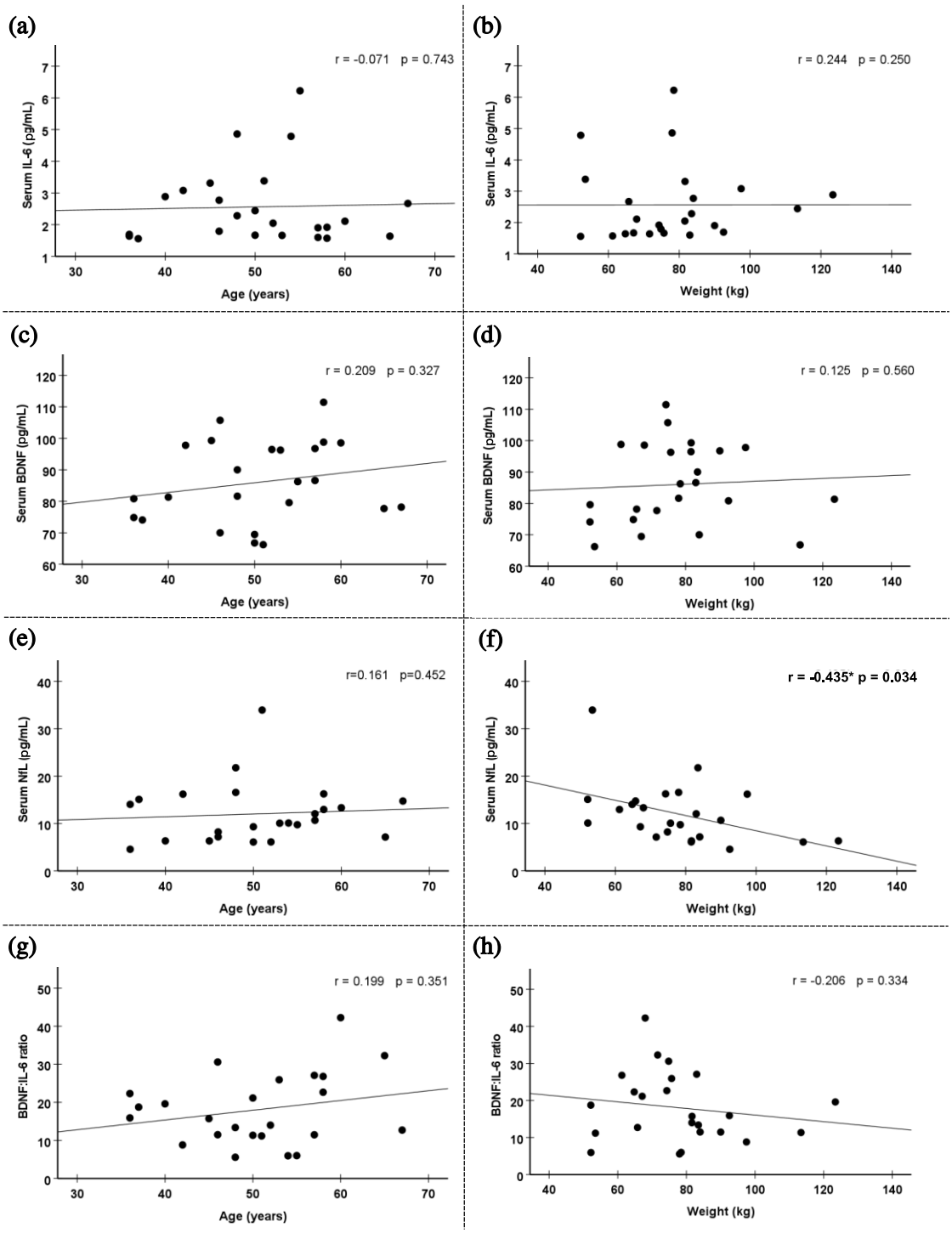

Figure 2.3. Relationships between biomarkers, age and weight

Data presented as individual values. (a) Relationship between Interlukin-6 and age; (b) Relationship between Interlukin-6 and weight; (c) Relationship between BDNF and age; (d) Relationship between BDNF and weight; (e) Relationship between NfL and age; (f) Relationship between NfL and weight; (g) Relationship between BDNF/IL-6 ratio and age; (h) Relationship between BDNF/IL-6 ratio and weight.

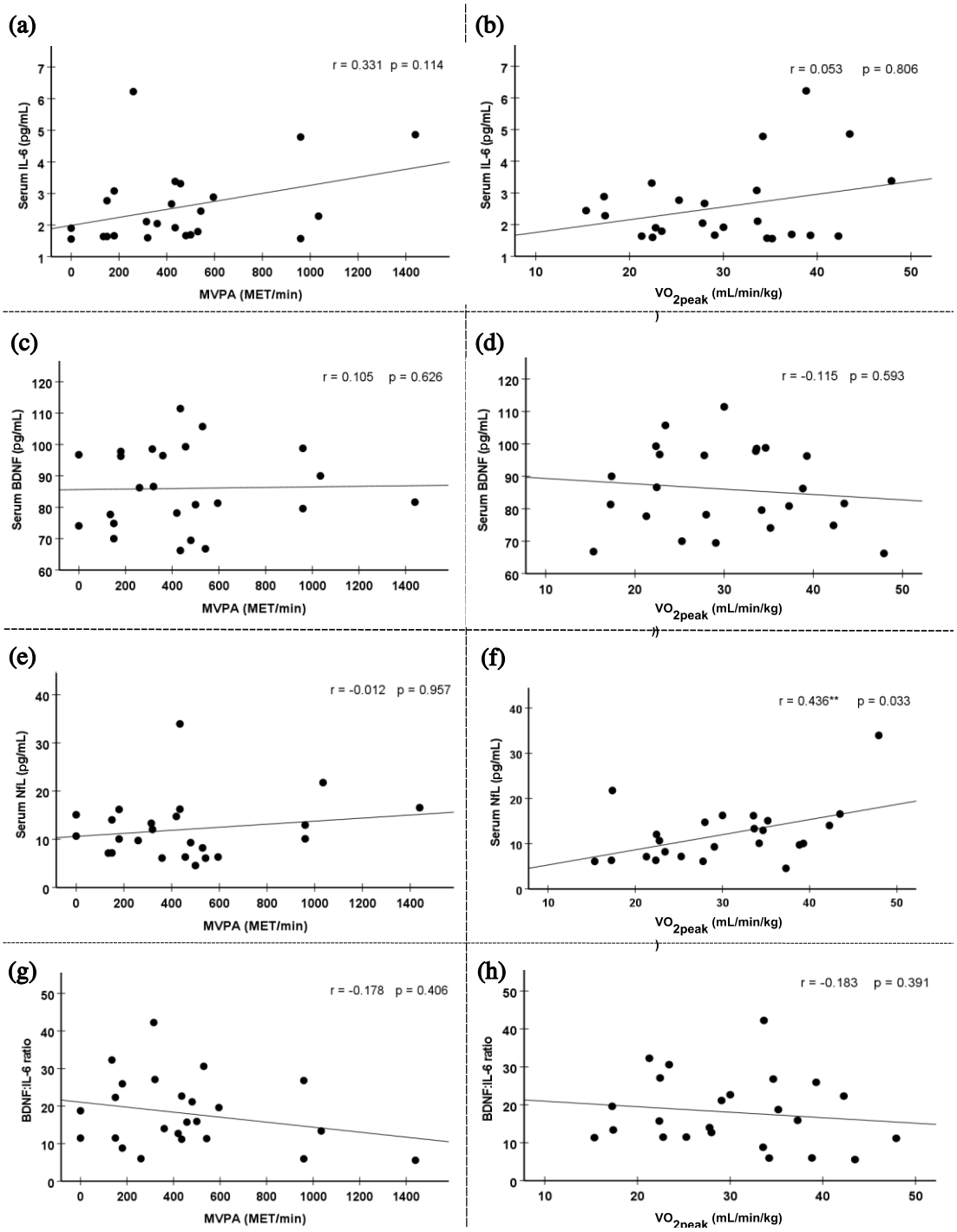

Figure 2.4. Relationships between biomarkers, VO_{2peak} (Fitness) and Moderate to Vigorous Physical Activity (MVPA)

Data presented as individual values. (a) Relationship between IL-6 and MVPA; (b) Relationship between IL-6 and VO_{2peak}; (c) Relationship between BDNF and MVPA; (d) Relationship between BDNF and VO_{2peak}; (e) Relationship between NfL and MVPA; (f) Relationship between NfL and VO_{2peak}; (g) Relationship between BDNF/IL-6 ratio and MVPA; (h) Relationship between BDNF/IL-6 ratio and VO_{2peak}.

2.5 Discussion

The need for efficient rehabilitation strategies for people with PMS is of paramount importance. Recent studies have mentioned that physical exercise may be a neuroprotective agent in MS because it seems to influence the axis of the neuro immune (Diechmann et al., 2021; Giesser, 2015). The progressive neurodegeneration and inflammation characteristic of MS might be balanced or normal by an active life that would include regular physical exercise.

Our investigation delved into the correlations between inflammatory (IL-6) and neurodegeneration (NfL) biomarkers, as well as indicators of neural repair (BDNF), with lifestyle factors such as exercise intensity and aerobic fitness. The study also explored the relationship of these biomarkers with variables like age and body mass. Research highlights that NfL levels in serum may serve as a key biomarker for tracking neurodegeneration during aging, aiding in distinguishing between typical aging and pathological brain alterations (Khalil et al., 2020). Moreover, the prognostic value of serum NfL in MS has been corroborated, suggesting its utility in tailoring individual disease management and treatment strategies (Benkert et al., 2022).

We report four main findings. First, the cytoskeleton protein NfL was present in serum in this sample suggesting that inflammation and neuronal damage was actively taking place, despite the fact that we ensured participants were free from relapses in the previous 3 months. Second, higher serum NfL was related to lower body weight. Third, we found that higher serum NfL was related to higher fitness, which seemed counterintuitive. Finally, there were no relationships between IL-6, BDNF, or BDNF/IL-6 ratio and age, weight, physical activity, or cardiorespiratory fitness.

In this light, these studies have broadened the complex interplay between physical activity, cardiorespiratory fitness, and biomarkers of neurodegeneration and inflammation in MS. Thus, NfL becomes promising for tracking the disease course and response to lifestyle interventions; however, the role of IL-6 and BDNF in this context remains to be determined. Our study's results substantiate the importance of NfL as a biomarker in the analysis of future studies and projects. There were no significant findings regarding the links between these biomarkers and lifestyle factors. Further research must be done to disentangle the pathophysiologic mechanisms that give rise to these relations. In addition, forthcoming studies must explore how the intensity and mode of physical activity alter IL-6 and BDNF levels to determine whether these varying factors can invariably translate into measures of clinical utility in MS. In summary, more research is necessary, while emphasizing the need for more nuanced investigations into the effects of lifestyle interventions on IL-6 and BDNF levels. Integrating findings from these studies, such as those by Khalil et al. (2020) and Benkert et al. (2022), with longitudinal and mechanistic research would be crucial to developing personalized, evidence-based strategies for managing MS through lifestyle modifications.

2.5.1 NfL Protein in Serum Indicates Ongoing Inflammation in MS Patients without Recent Relapses

MS is characterized by the immune system's attack on the myelin sheath surrounding nerve fibers, resulting in inflammation, demyelination, and axonal damage (Høglund & Maghazachi, 2014). In recent research involving 24 MS participants, we explored the potential of NfL as a reliable biomarker for assessing neuronal damage in this context. NfL is a protein that is released into both the cerebrospinal fluid and the bloodstream when neurons undergo damage. This marker

has shown promise in detecting neuronal damage across various neurodegenerative diseases and other neurological impairments (Barro et al., 2020). Studies have shown that serum NfL levels can be elevated in MS patients even in the absence of clinical relapses, suggesting ongoing subclinical disease processes (Kuhle et al., 2019; Disanto et al., 2017). This is particularly relevant for monitoring disease progression and treatment response in MS patients. Research indicates that serum NfL levels differ across MS subtypes and are higher in patients with PMS compared to relapsing-remitting MS, aligning with the greater degree of neurodegeneration typically seen in progressive forms of the disease (Barro et al., 2020). The dynamics of NfL levels in relation to physical fitness and lifestyle factors have been a subject of interest. Some studies suggest that exercise may have a modulatory effect on NfL levels, potentially reflecting neuroprotective mechanisms (Barro et al., 2020; Khalil et al., 2020; Thebault et al., 2020).

To further contextualize our findings, recent studies provide invaluable insights into the utility of NfL as a biomarker for neurodegeneration and disease progression. A study on individual prognostication of disease activity in MS patients highlighted that NfL levels, adjusted for age and BMI, could predict future disease activity, underscoring its potential as a valuable biomarker even without clinical symptoms. This supports our observation of elevated NfL levels among our participants, suggesting a subclinical progression of neuronal damage (Benkert et al., 2022). In conclusion, NfL levels represent an important biomarker for ongoing inflammation and neurodegeneration in MS, independent of relapse activity. As such, they offer a valuable tool for clinicians to monitor disease progression and potentially guide therapeutic interventions.

2.5.2 Serum NfL Levels and Lower Body Weight in MS Participants

The dynamics of the relationship between body weight, blood volume, and the concentrations of NfL in the context of MS are still under interrogation and development. While this area is poorly explored, since the description of the pathophysiology of MS increased to great depth, more studies have been conducted on how physiological factors of weight and blood volume affect NfL concentrations. In early research, such as by Yalachkov et al. (2022), results proposed that body mass index and estimated blood volume may exert an effect on measurements of serum NfL, possibly from alterations in the volume of distribution. This suggests an added aspect of complexity that complicates our understanding of NfL dynamics, compared to a study by Manouchehrinia et al. (2020), which did not observe any significant relationship between body metrics and cerebrospinal fluid NfL levels after adjustments for demographic variables. Moreover, the review by Barro et al. (2020) emphasizes the importance of considering extraneural factors such as body mass index while interpreting the blood NfL concentrations.

Our investigation contributes to the evolving narrative by uncovering a notable association between lower body weight and elevated NfL levels in individuals with MS. This discovery prompts a broader examination of the factors influencing serum NfL concentrations, which include demographic, lifestyle, and comorbidity variables. The literature identifies several determinants of NfL levels, such as exercise, blood volume, age, and body mass index, with aging notably associated with increased NfL, reflecting the natural progression of brain aging and the acceleration of neuronal injury in older populations (Benkert et al., 2022; Hermesdorf et al., 2022; Joisten et al., 2021; Khalil et al., 2020; Vågberg et al., 2015). Conversely, the phenomenon of decreased NfL levels in obese individuals, as discussed by Hermesdorf et al. (2022), introduces a

counterintuitive aspect to the relationship between NfL concentrations and body weight, suggesting a dilution effect attributable to higher blood volume in obese populations.

However, this relationship is nuanced in the context of obesity in a healthy population, where it has been observed that obese individuals may have decreased NfL levels due to their higher blood volume (Hermesdorf et al., 2022). This suggests that while NfL levels increase with age, they may decrease in obese individuals due to the dilution effect of higher blood volume. These results highlight the complex interplay between age and NfL levels, underscoring the need for further research to understand their relationship entirely.

Our findings suggest that blood NfL levels could be useful as a valuable biomarker for detecting ongoing inflammation and neuronal damage in individuals with MS, even without clinical symptoms. The association between serum NfL levels and reduced body weight in MS individuals further underscores the intricate interplay between NfL and neuronal damage. The precise mechanism behind this intriguing link remains a subject for further investigation and necessitates additional research. Moreover, the findings from studies examining NfL levels in normal aging elucidate the biomarker's increase with age and its association with morphological brain changes, indicating an acceleration of neuronal injury in older populations (Khalil et al., 2020). These observations align with our report of higher serum NfL related to lower body weight, suggesting that the relationship between NfL levels and body weight might also be influenced by age-related neurodegenerative processes, thereby highlighting a multifaceted relationship that warrants comprehensive future studies to unravel the complex dynamics at play.

2.5.3 Higher Cardiorespiratory Fitness Linked to Elevated Serum NfL in MS

In our recent investigation, we unearthed a noteworthy correlation between cardiorespiratory fitness, as measured by VO_{2peak}, and NfL levels in individuals with MS. This intersection of NfL levels and cardiorespiratory fitness has become an increasingly vital focal point within MS research, with NfL emerging as a potential biomarker signifying neurodegenerative processes in MS. Elevated NfL levels are indicative of more extensive neuronal damage, while the pursuit of overall health and mobility in individuals with MS necessitates the maintenance of cardiorespiratory fitness, typically assessed using $VO_{2\ peak}$ as a key metric (Motl & Baird, 2021; Varhaug et al., 2019; Yang et al., 2022).

Contrary to initial assumptions, our findings suggest that higher cardiorespiratory fitness is linked to elevated serum NfL in MS participants. This observation is intriguing, given that previous research indicated increased NfL levels in MS participants are linked to lower levels of cardiorespiratory fitness, as shown by lower $VO_{2\ peak}$ values (Ercan et al., 2021). This rise in NfL levels aligns with a decline in cardiorespiratory fitness attributed to persistent neuronal damage, shedding light on the intricate interplay between neurological and physiological elements within the context of MS. The development of therapies aimed at preserving or enhancing cardiorespiratory health among this population may, therefore, be guided by the monitoring of NfL levels, serving as a valuable tool for assessing the risk of declining fitness in individuals with MS (Cruickshank et al., 2020; Ercan et al., 2021).

To tailor specialized rehabilitation and exercise regimens for those afflicted with MS, a comprehensive understanding of the interrelationship between NfL and cardiorespiratory fitness is imperative. The potential for reducing chronic systemic low-grade inflammation, a hallmark of MS, through progressively challenging aerobic training aimed at improving fitness or physical

activity status, emerges as an intriguing prospect. Moreover, cross-sectional research among healthy older individuals has unearthed a connection between the level of physical activity and NfL concentrations, with higher physical activity levels linked to lower NfL levels (Beavers et al., 2010; Raffin et al., 2021).

The existing literature underscores the relationship between serum NfL concentration and various facets of MS, including disease activity, diagnosis, neurodegeneration, and disease progression (Bridel et al., 2019; Kapoor et al., 2020). Discrepancies in biomarker concentrations across studies can be attributed in part to differences in measurement techniques, sampling times, and participants' emotional states. Additionally, a separate study found that NfL and GFAP levels in the experimental group significantly decreased after the trial, with no significant changes observed in the control group; notably, the ΔNfL levels of the experimental group surpassed those of the control group (Ercan et al., 2021). Conversely, research focused on individuals with mild Alzheimer's disease did not establish a discernible connection between aerobic exercise and NfL (Frederiksen et al., 2023).

In summary, our investigation uncovers a compelling link between NfL levels and cardiorespiratory fitness in individuals grappling with MS, marking a significant stride in understanding the intricate dynamics of this debilitating condition. The prospect of harnessing NfL as a tool to gauge and manage cardiorespiratory fitness decline within the MS population opens promising avenues for future interventions. As we delve deeper into the interplay between neurological biomarkers and physical well-being, we inch closer to more effective strategies for alleviating the impact of MS-related neurodegeneration on cardiorespiratory fitness. Additionally, the variability in biomarker levels between studies underscores the importance of standardizing

measurement techniques and considering contextual factors, shedding light on the complex landscape of biomarker research within neurological disorders like MS.

2.5.4 IL-6, BDNF, and BDNF/IL-6 Ratios Unaffected by Age, Weight, Physical Activity, or Cardiorespiratory Fitness in MS Participants

In the pursuit of understanding the intricate relationship between exercise, neuroinflammatory diseases like MS, and the levels of crucial biomarkers such as BDNF and IL-6, our study embarked on a comprehensive investigation. The aim was to shed light on whether physical activity and cardiorespiratory fitness levels might hold the key to managing and potentially mitigating the effects of MS.

Our study involved participants who underwent Graded Exercise Testing using a recumbent stepper until they reached the point of maximal voluntary exertion. We found no relation in serum BDNF levels in response to the exercise, participants exhibited notably poor levels of cardiorespiratory fitness, which could explain the lack of significant BDNF changes. Here are some studies that show a positive result but had better fitness in their sample. This outcome led us to consider the research of Cabral-Santos et al. (2016) and Rasmussen et al. (2009), who found that the release of BDNF in the blood is inversely correlated with exercise intensity. However, it's worth noting that our study's participants exhibited notably poor levels of cardiorespiratory fitness, which could explain the lack of significant BDNF changes. In contrast, Gold et al. (2003) reported a significant increase in serum BDNF levels after exercise. However, their study involved participants with different demographic profiles than our sample. Their age were lower (39.2 ± 1.8 in their sample; 50.46 ± 8.55 in our sample), their disease duration was shorter (10.5 ± 1.5 years in their sample; 17.5 ± 7.7 years in our sample) and their mean weight

was greater (69.5 ± 2.3 kg in their sample; 77.84 ± 17.44 in our sample). Briken et al. (2016) also observed a BDNF increase in a sample of MS patients with moderate impairment after a shorter exercise duration. These divergent results underline the complexity of the relationship between exercise and BDNF in the context of MS. Additionally, (Devasahayam et al., 2021) highlighted the importance of the BDNF/IL-6 ratio in reflecting immune system efforts and their potential implications for improving the well-being of MS patients Consequently, further research is imperative to elucidate the delicate balance between inflammation and repair in neuroinflammatory diseases like MS.

Another significant observation in our study was the absence of correlations between age, weight, physical activity, and cardiorespiratory fitness in MS individuals with IL-6, BDNF, and BDNF/IL-6 ratios. This intriguing lack of connection might be attributed to various factors, including the possibility that these biomarkers are not directly linked to the progression and severity of MS. Genetics, environmental factors, or comorbidities could potentially exert a stronger influence (Ghasemi et al., 2017; Tafti et al., 2022). Additionally, a thorough investigation and meta-analysis was conducted by (Shobeiri, Seyedmirzaei, et al., 2022) indicated that physical activity did not induce changes in IL-6 levels in MS patients. These results raise questions about the connection between IL-6 and physical activity in individuals with MS, warranting further exploration. Previous research has suggested that biomarkers like IL-6 and BDNF may be linked to MS progression and severity, emphasizing the importance of unraveling their intricate roles (Briken et al., 2016; Devasahayam et al., 2021; Nociti et al., 2022).

Moreover, research into the connection between serum neurotrophins and maximal aerobic exercise in stroke patients revealed interesting insights. (King et al., 2019) discovered that, following exercise, BDNF levels remained unchanged, but they did observe a positive relationship

between height and resting BDNF levels. This finding, coupled with their observation that higher resting BDNF was associated with less physical activity and younger age, suggests that various factors interact in shaping BDNF levels. These findings extend the intricate web of BDNF's responses to exercise and underscore the need for considering factors like age, physical activity, and disability severity in such investigations (King et al., 2019).

Recent studies have illuminated the potential of exercise to protect the brain from the ravages of MS through interactions with the neuroimmune axis (Alvarenga-Filho et al., 2016; Motl & Sandroff, 2018). This growing body of evidence points toward physical activity and fitness as potential avenues for managing MS. Nonetheless, a comprehensive understanding of this relationship remains elusive, emphasizing the necessity of ongoing research in this field.

Results from earlier studies suggested that mood disorders may be influenced by an imbalance between pro- and anti-inflammatory cytokines, as indicated by a greater ratio of tumor necrosis factor/IL-10 and IL-6/IL-10 in the brains of rats subjected to chronic mild stress (You et al., 2011). In a study of 66 recipients of hematopoietic stem cell transplants, those with depression had greater IL-6/IL-10 ratios than controls (Tavakoli-Ardakani et al., 2015). Exercise is thought to cause the release of IL-6 from skeletal muscle, which is thought to suppress tumor necrosis factor, a key mediator of inflammation, in healthy volunteers (Starkie et al., 2003). In a study by (Gravesteijn et al., 2023), they did not find changes in NfL , sGFAP and sBDNF.

As previously demonstrated, BDNF's reaction to exercise training is determined by sociodemographic factors including body composition (Dinoff et al., 2016). Weight gain appears to be linked to low-grade inflammation (Heilbronn & Campbell, 2008), which can inhibit the generation of neurotrophic factors including platelet-derived growth factor and BDNF (Mokhtarzade et al., 2018). In a systematic review and meta-analysing paper they demonstrated

that physical activity increases the BDNF's levels in individuals with MS but they did not find any significant relationship with sex and age (Shobeiri, Karimi, et al., 2022). This study reported no significant relationship between confounders and blood biomarkers, but in our study, we found a significant relationship between weight and NfL (R= -0.498, P= 0.011).

Mokhtarzade et al. (2017) reported that weight loss following exercise is associated with an increase in the level of anti-inflammatory cytokines such as IL-10 and adiponectin in person with exercise-induced weight loss is linked to an increase in IL-10 and adiponectin levels, two anti-inflammatory cytokines, in individuals with MS. Therefore, weight reduction and its role in the impact of exercise on cytokines and adipokines in MS should receive special consideration in future investigations. In a study among people with progressive multiple sclerosis there were no statistically significant variations in age, sex distribution, or body mass index between the MS group and controls (Devasahayam et al., 2021).

Gravesteijn et al. (2023) demonstrated, after a 16 weeks high intensity aerobic exercise training program, did not find any significant relationship between biomarkers (BDNF and NfL) and age. Briken et al. (2016), reported no significant changes after 22 sessions of training in 42 patients with progressive MS; in addition, acute or extended exercise had no appreciable impact on irisin or interleukin-6 (Devasahayam et al., 2021). showed that IL-6 in MS subjects in the exercise and resting group were higher than control group. In (Devasahayam et al., 2021) study, participants with progressing MS in the untrained trial group reported a 40% increase in blood IL-6 levels following the Graded Exercise Testing. Recent studies have provided evidence to suggest that exercise may confer neuroprotection in individuals with MS through its interactions with the neuro-immune axis (Diechmann et al., 2021; Langeskov-Christensen et al., 2017; Motl & Sandroff, 2018).

In summary, our study investigated the relationships between exercise, neuroinflammatory diseases such as MS, and key biomarkers such as BDNF and IL-6. We did not find a significant increase in BDNF associated with exercise in MS patients, which is consistent with some research in the more general research context. Our findings indicate that NfL levels are significantly associated with body weight and physical fitness; therefore, these biomarkers could be used to indicate subclinical neurodegeneration and the putative role of lifestyle factors.

The role of IL-6 and BDNF in neuroprotection and repair in MS remains complex and multifaceted. Exercise seems to have variable effects on these biomarkers, depending on exercise intensity, exercise duration, and probably individual patient characteristics. This complexity underlines the need for further research to unravel these relationships and understand how exercise modulates neuroinflammatory processes. Our findings point to a promising neuroprotective role of exercise in MS but indicate that the path ahead requires careful consideration of the interaction between various biomarkers, physical fitness, and other variables. Continued research is needed to improve the quality of life and to develop possible therapeutic strategies and for individuals suffering from neuroinflammatory diseases.

2.5.5 Limitations

The limitations of our investigation must be acknowledged. Our findings' generalizability is constrained by the cross-sectional design and small sample size. Future studies with longitudinal designs and larger sample sizes are warranted to further explore the relationships between physical activity, cardiorespiratory fitness, and blood biomarkers in individuals with MS. We are aware that the absence of a control group of participants may be one of our study's limitations. We also can

mention the time of blood sampling and the participant's mood which may be different among participants and could affects the levels of the biomarkers.

Only one blood testing was conducted; for future research studies we suggest having 2-time blood testing, before and after the program. We also had participant physical activity self-reports which may be lacking in accuracy. Finally, IL-6 is a pleiotropic cytokine that plays a role in a number of biological processes, including inflammation, metabolism, oncogenesis, immune regulation, and hematopoiesis (Kang et al., 2019). The interpretation of IL-6-related data from our investigation must be restricted to the particular setting of our research because IL-6 can participate in numerous, potentially overlapping signaling processes.

2.6 Conclusion

In conclusion, our study suggests that in individuals with MS, physical activity and cardiorespiratory fitness may not directly influence blood biomarkers associated with neuroinflammation and repair, as measured by BDNF, NfL, and IL-6. Nonetheless, it is important to highlight that these findings are derived from a limited sample size, and additional research is required to verify our outcomes. The lack of significant relationships with BDNF and IL-6 levels could be due to several factors, including the variability in exercise intensity, duration, and individual patient characteristics. Additionally, NfL levels, while correlated with physical fitness and body weight, did not show a relationship with moderate to vigorous physical activity. Future studies should explore additional biomarkers and employ longitudinal designs to gain a better understanding of the complex relationships between physical activity, cardiorespiratory fitness, and blood biomarkers in individuals with MS. Overall, we can draw the conclusion that more study

is necessary to clarify the relationship between physical activity and cardiorespiratory fitness and biomarker levels.

2.7 Source of Funding

The study was supported by the Canadian Institutes of Health Research (CIHR to MP 173526), and the School of Graduate Studies, Memorial University.

2.8 Conflicts of Interest

The authors report no conflicts.

3 Chapter Three: Discussion

3.1 Overview/ Key Findings

Multiple sclerosis (MS) presents a complex challenge, necessitating nuanced approaches to behavioral and rehabilitative strategies (Proschinger et al., 2022). Current recommendations endorse a moderate to vigorous degree of physical activity, including frequent exercise, as a potential intervention (Motl et al., 2022; Sandroff et al., 2012), and the beneficial effects of exercise on neuroinflammation and brain repair have been well-documented in various neurological conditions (Mee-Inta et al., 2019; Svensson et al., 2015). Our study aimed to investigate the connections between physical activity, cardiorespiratory fitness, and blood biomarkers associated with neuroinflammation and repair in individuals with MS. We investigated whether cardiorespiratory fitness (VO_{2peak}) and self-reported physical activity were related to blood biomarkers of neuroinflammation (NfL and IL-6) and repair (BDNF) in individuals with MS.

We demonstrated the monitoring and predictive value of the cytoskeleton protein NfL. The levels of the biomarker were detected in serum of participants with a three-month relapse free course of MS suggesting that inflammation and neuronal damage was actively taking place, despite absence of objective signs and symptoms of the disease progression. We assessed the relationships between moderate-to-vigorous physical activity (MVPA), cardiorespiratory fitness, age, weight, and various biomarkers (BDNF, IL-6, NfL, and BDNF/IL-6 ratio). The results indicated a correlation between serum NfL levels and weight and VO_{2peak} ($r= -0.435$, $p= 0.034$, and $r= 0.436$, $p= 0.033$), while the other biomarkers did not show significant correlations with MVPA, cardiorespiratory fitness, age, or weight ($p > 0.05$). Our results suggest that higher serum NfL, was related to lower body weight and higher cardiorespiratory fitness (VO_{2peak}). The correlations

indicate that the elevated levels of the pro-inflammatory biomarker are linked to individuals with MS who maintain a lower body weight and exhibit better physical fitness. A number of confounding factors, such as BMI (Benkert et al., 2022), blood volume, body fat mass, and total body water (Hermesdorf et al., 2022), medication, smoking, and time since relapse, not considered in our study, might have contributed to the results. This interesting observation highlights the role of weight, cardiorespiratory fitness, age, and effects of medication in regulating inflammatory processes seen in MS yet requires further research and careful interpretation.

In this limited sample of MS patients, BDNF, IL-6, and the BDNF/IL-6 ratio exhibited no discernible changes based on physical activity, cardiorespiratory fitness level, age, or body weight. It appears that BDNF and IL-6 may lack sensitivity in detecting relationships induced by cardiorespiratory fitness and MVPA in this small patient sample. Despite this, our findings enhance our understanding of the relationship between biomarkers and physical activity in the MS population. In conclusion, this study significantly contributes to our knowledge of specific biomarker correlations with physical activity and cardiorespiratory fitness in MS. However, the limitations in the responsiveness of BDNF, IL-6, and the BDNF/IL-6 ratio highlight the necessity for further investigations. These studies can deepen our understanding of the challenges associated with using specific biomarkers in multiple sclerosis populations, emphasizing the importance of gaining insights to enhance interventions for individuals with MS.

3.2 Limitations/Challenges

Our study has several limitations that must be acknowledged. First, the nature of our cross-sectional study design makes it more difficult to derive causal relationships between the parameters from analysis, as we measure the outcome and the exposures in the participants at the same time.

Second, our study included only 24 participants with two phenotypes of MS combined and no control group, which makes interpretation of the biomarker levels more challenging. Third, an assessment of physical activity requires a higher level of precision. In our study MVPA was assessed by the adapted International Physical Activity Questionnaire (IPAQ). The questionnaire lists all types of physical activities only during previous 24 hours before the visit, and relies on a self-reported values in minutes. This approach might underestimate or overestimate the objective regular level of physical activity of a particular participant and alter the direction and strength of correlations.

Besides, the presence of concomitant diseases or medical conditions may influence the results of the blood tests and cardiorespiratory fitness tests. We did not collect dietary preferences, alcohol consumption, current smoking status, presence of sleep disturbances, physical activity pattern information, presence of concomitant cardiovascular or pulmonary diseases, disturbances in glucose metabolism from participants to see if these factors had any influence on the measurement of blood biomarkers, especially BDNF, IL-6, and VO_{2peak}. Medications may have had a significant impact on our results. Modern treatments slow down the progression of MS by limiting the immune response and decreasing inflammation, which influences the levels of biomarkers in the systemic circulation. NfL, for instance, is considered to be not only diagnostic and prognostic biomarker, but a monitoring biomarker as shown in our study. So, levels of NfL may vary depending on the treatment strategy and a medication used.

Furthermore, it is worth noting that sex differences could explain the obtained results since male and female populations are inherently different in weight and VO_{2peak}. Although our present study is not powered enough to test for sex differences, future studies could either exclude males completely or test males and females separately to determine if our results hold in a more

homogeneous female sample. Of specific value would be to make it evident if the relationship between NfL levels, weight, and VO_{2peak} indicates a sex difference for this biomarker. By addressing this in future research, we can ensure more robust and tailored findings, providing valuable insights for personalized MS management.

Despite these limitations, this study has provided additional information about the connections between the level of NfL, weight, and cardiorespiratory fitness in patients with MS. Further research is necessary to address these limitations and provide a more comprehensive understanding of the role of blood biomarkers and their relationship with physical activity and cardiorespiratory fitness in individuals with MS. The information from our study might be useful in designing a cohort study including more participants with one phenotype on a stable treatment, comparing the results with matching healthy controls or utilizing the longitudinal design with planned intervention.

In conducting an extensive inquiry into the intricate associations between biomarkers and crucial health indicators in individuals diagnosed with MS, our study represents a noteworthy contribution, offering valuable insights into the nuanced interrelationships within this intricate domain. More precisely, our research illuminates the prospective utility of NfL as a promising indicator for evaluating inflammation in the context of body weight and physical fitness. Nevertheless, our findings highlight the imperative for further expansive investigations to clarify the responsiveness of additional scrutinized biomarkers to these factors within this distinctive population.

3.3 Recommendations for Future Study

3.3.1 Longitudinal Study Design and Multiple Blood Testing

A shift towards longitudinal study designs with planned intervention is recommended for future investigations. Longitudinal studies, tracking participants over an extended period, offer a more dynamic understanding of how biomarkers respond to sustained physical activity and changes in cardiorespiratory fitness levels. This approach facilitates the identification of patterns and trends, providing a more comprehensive view of the explored relationships. Additionally, implementing two rounds of blood testing – both before and after interventions – would provide a more comprehensive understanding of the temporal changes in biomarkers in response to physical activity and cardiorespiratory fitness interventions. This approach enables a nuanced analysis of how these markers may dynamically respond over time, providing insights into the sustainability of observed effects.

3.3.2 Concomitant Diseases and Risk Factors

BDNF is an anti-inflammatory biomarker and its level in peripheral circulation may be influenced by a number of medical conditions and risk factors. Low levels of BDNF had been detected in previous studies in patients with obesity, anxiety, depression, and diabetes mellitus (Fujinami et al., 2008; Krabbe et al., 2007; Li et al., 2016; Moosaie et al., 2023). Besides, the level might be greatly influenced by stress and estrogens (Eckert et al., 2017; Hosang et al., 2014). Thus, collecting additional information on the factors or conditions that may influence the level of BDNF is crucially important for further research.

IL-6 is a cytokine with multiple functions, including immune response and hematopoiesis. The signaling molecule plays a central role in the stimulation of acute phase responses, has a pro-atherogenic effect, and promotes insulin resistance. The mean age of the participants in our study was 50.46(±8.55) years, which is an independent risk factor that increases the possibility of developing cardiovascular diseases, obesity and diabetes mellitus. So, accounting for those conditions is highly important for the correct interpretation of the blood test results for IL-6. Besides, smoking is also a potent activator of white blood cells, leading to increased production of IL-6 and systemic inflammation (Liu et al., 2023; McEvoy et al., 2015) and smoking status should be included in the analysis and interpretation of IL-6 levels in future research.

3.3.3 Effect of Pharmacological Treatment

Further researchers should consider the impact of disease-modifying therapies on the progression of MS. Modern medications limit the inflammatory response, influencing the levels of pro-inflammatory and anti-inflammatory cytokines as well as their ratios. Our study showed no relationships between IL-6, BDNF, or BDNF/IL-6 ratio and age, weight, physical activity, or cardiorespiratory fitness. The results could be influenced by the treatment regimen or no treatment in people with very mild symptoms. Thus, evaluation of pharmacologic treatment is recommended for the further studies. Understanding how medications may influence biomarker responses is crucial for untangling the complexities of these relationships and refining the specificity of exercise-based interventions for individuals with MS.

3.3.4 Precise Assessment of Regular Physical Activity in MS Participants

The use of more precise methods of physical activity monitoring may have a significant impact on the results of future research. Moderate to vigorous physical activity, determined by the International Physical Activity Questionnaire in our study, did not show any significant correlations with age, weight and blood biomarkers. However, utilization of tri-axial accelerometers, heart rate monitors or pedometers may give much more objective information about the level of physical activity of a particular individual over a period of time, eliminating a subjective component from the data. The results of objective measures might be more effective in discovering the presence of connection between physical activity and biomarkers.

3.4 Conclusion

In conclusion, this study contributes valuable insights into the intricate relationships between biomarkers and critical health parameters in individuals with MS. While the correlation of cardiorespiratory fitness and weight with NfL suggests promising avenues for assessing inflammation and impact of physical activity, the limited responsiveness of other investigated markers emphasizes the need for caution in interpretation. The study, conducted in a small sample, highlights the complexity of these relationships and emphasizes the necessity for more extensive research. Given the ongoing development of our comprehension of the bond between exercise and these biomarkers, it is imperative to conduct additional investigations utilizing larger and more diverse cohorts to uncover the intricate mechanisms involved and improve our ability to manage MS through exercise-based interventions.

References

Abbaspoor, E., Zolfaghari, M., Ahmadi, B., & Khodaei, K. (2020). The effect of combined functional training on BDNF, IGF-1, and their association with health-related fitness in the multiple sclerosis women. *Growth Hormone & IGF Research*, *52*, 101320. https://doi.org/ 10.1016/j.ghir.2020.101320

ACoS, M. (2013). *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins.

Ainsworth, B., Cahalin, L., Buman, M., & Ross, R. (2015). The current state of physical activity assessment tools. *Progress in cardiovascular diseases*, *57*(4), 387-395. https://doi.org/10.1016/j.pcad.2014.10.005

Adamson, B. C., Ensari, I., & Motl, R. W. (2015). Effect of exercise on depressive symptoms in adults with neurologic disorders: a systematic review and meta-analysis. *Archives of physical medicine and rehabilitation*, *96*(7), 1329-1338. https://doi.org/10.1016/j.apmr.2015.01.005

Alcolea, D., Beeri, M. S., Rojas, J. C., Gardner, R. C., & Lleó, A. (2023). Blood Biomarkers in Neurodegenerative Diseases: Implications for the Clinical Neurologist. *Neurology*. https://doi.org/10.1212/WNL.0000000002071

Alharbi, F. M. (2015). Update in vitamin D and multiple sclerosis. *Neurosciences Journal*, *20*(4), 329-335. https://doi.org/10.17712/nsj.2015.4.20150357

Alvarenga-Filho, H., Sacramento, P. M., Ferreira, T. B., Hygino, J., Abreu, J. E. C., Carvalho, S. R., Wing, A. C., Alvarenga, R. M. P., & Bento, C. A. (2016). Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple sclerosis patients in response to neuromediators. *Journal of neuroimmunology*, *293*, 91-99. https://doi.org/10.1016/j.jneuroim.2016.02.014

Amatya, B., Khan, F., & Galea, M. (2019). Rehabilitation for people with multiple sclerosis: an overview of Cochrane Reviews. *Cochrane Database of Systematic Reviews*(1). https://doi.org/10.1002/14651858.CD012732.pub2

Angst, J., & Merikangas, K. (1997). The depressive spectrum: Diagnostic classification and course. *Journal of Affective Disorders*, *45*(1), 31–40. https://doi.org/10.1016/S0165-0327(97)00057-8.

Arena, R., Myers, J., Williams, M. A., Gulati, M., Kligfield, P., Balady, G. J., Collins, E., & Fletcher, G. (2007). Assessment of functional capacity in clinical and research settings: a scientific statement from the American Heart Association Committee on Exercise, Rehabilitation, and Prevention of the Council on Clinical Cardiology and the Council on Cardiovascular Nursing. *Circulation*, *116*(3), 329-343. https://doi.org/10.1161/CIRCULATIONAHA.106.184461

Arslan, B., & Zetterberg, H. (2023). Neurofilament light chain as neuronal injury marker–what is needed to facilitate implementation in clinical laboratory practice? *Clinical Chemistry and Laboratory Medicine (CCLM)*, *61*(7), 1140-1149. https://doi.org/10.1515/cclm-2023-0036

Axelsson, M., Malmeström, C., Gunnarsson, M., Zetterberg, H., Sundström, P., Lycke, J., & Svenningsson, A. (2014). Immunosuppressive therapy reduces axonal damage in progressive multiple sclerosis. *Multiple Sclerosis Journal*, *20*(1), 43-50. https://doi.org/10.1177/1352458513490544

Azoulay, D., Urshansky, N., & Karni, A. (2008). Low and dysregulated BDNF secretion from immune cells of MS patients is related to reduced neuroprotection. *Journal of neuroimmunology*, *195*(1-2), 186-193. https://doi.org/10.1016/j.jneuroim.2008.01.010

Bai, Z., Chen, D., Wang, L., Zhao, Y., Liu, T., Yu, Y., Yan, T., & Cheng, Y. (2019). Cerebrospinal fluid and blood cytokines as biomarkers for multiple sclerosis: a systematic review and meta-analysis of 226 studies with 13,526 multiple sclerosis patients. *Frontiers in Neuroscience*, *13*, 1026. https://doi.org/10.3389/fnins.2019.01026

Baird, J. F., Sandroff, B. M., & Motl, R. W. (2018). Therapies for mobility disability in persons with multiple sclerosis. *Expert review of neurotherapeutics*, *18*(6), 493-502. https://doi.org/10.1080/14737175.2018.1478289

Banitalebi, E., Ghahfarrokhi, M. M., Negaresh, R., Kazemi, A., Faramarzi, M., Motl, R. W., & Zimmer, P. (2020). Exercise improves neurotrophins in multiple sclerosis independent of disability status. *Multiple sclerosis and related disorders*, *43*, 102143. https://doi.org/10.1016/j.msard.2020.102143Get rights and content

Bansi, J., Bloch, W., Gamper, U., & Kesselring, J. (2013). Training in MS: influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. *Multiple Sclerosis Journal*, *19*(5), 613-621. https://doi.org/10.1177/1352458512458605

Bar-Or, A., Banwell, B., Berger, J. R., & Lieberman, P. M. (2022). Guilty by association: Epstein–Barr virus in multiple sclerosis. *Nature Medicine*, *28*(5), 904-906. https://doi.org/10.1038/s41591-022-01778-3

Barro, C., Benkert, P., Disanto, G., Tsagkas, C., Amann, M., Naegelin, Y., Leppert, D., Gobbi, C., Granziera, C., & Yaldizli, Ö. (2018). Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. *Brain*, *141*(8), 2382-2391. https://doi.org/10.1093/brain/awy154

Barro, C., Chitnis, T., & Weiner, H. L. (2020). Blood neurofilament light: a critical review of its application to neurologic disease. *Annals of clinical and translational neurology*, *7*(12), 2508-2523. https://doi.org/10.1002/acn3.51234

Barry, A., Cronin, O., Ryan, A. M., Sweeney, B., O'Toole, O., O'Halloran, K. D., & Downer, E. J. (2019). Cycle ergometer training enhances plasma interleukin-10 in multiple sclerosis. *Neurological Sciences*, *40*, 1933-1936. https://doi.org/10.1007/s10072-019-03915-2

Barry, A., Cronin, O., Ryan, A. M., Sweeney, B., Yap, S. M., O'Toole, O., Allen, A. P., Clarke, G., O'Halloran, K. D., & Downer, E. J. (2016). Impact of exercise on innate immunity in multiple sclerosis progression and symptomatology. *Frontiers in Physiology*, *7*, 194. https://doi.org/10.3389/fphys.2016.00194

Bayles, M. P. (2023). *ACSM's exercise testing and prescription*. Lippincott Williams & Wilkins.

Beavers, K. M., Brinkley, T. E., & Nicklas, B. J. (2010). Effect of exercise training on chronic inflammation. *Clinica chimica acta*, *411*(11-12), 785-793. https://doi.org/10.1016/j.cca.2010.02.069

Becher, B., Spath, S., & Goverman, J. (2017). Cytokine networks in neuroinflammation. *Nature Reviews Immunology*, *17*(1), 49-59.

Beltran-Valls, M. R., Adelantado-Renau, M., & Moliner-Urdiales, D. (2018). Association between objectively measured physical activity and plasma BDNF in adolescents: DADOS study. *Journal of Molecular Neuroscience*, *65*, 467-471. https://doi.org/10.1007/s12031-018-1122-2

Benedict, C., Blennow, K., Zetterberg, H., & Cedernaes, J. (2020). Effects of acute sleep loss on diurnal plasma dynamics of CNS health biomarkers in young men. *Neurology*, *94*(11), e1181-e1189. https://doi.org/10.1212/WNL.0000000000008866

Benkert, P., Meier, S., Schaedelin, S., Manouchehrinia, A., Yaldizli, Ö., Maceski, A., Oechtering, J., Achtnichts, L., Conen, D., & Derfuss, T. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *The Lancet Neurology*, *21*(3), 246-257. https://doi.org/10.1016/S1474-4422(22)00009-6

Berkowitz, S., Achiron, A., Gurevich, M., Sonis, P., & Kalron, A. (2019). Acute effects of aerobic intensities on the cytokine response in women with mild multiple sclerosis. *Multiple sclerosis and related disorders*, *31*, 82-86. https://doi.org/10.1016/j.msard.2019.03.025

Bhan, A., Jacobsen, C., Myhr, K. M., Dalen, I., Lode, K., & Farbu, E. (2018). Neurofilaments and 10-year follow-up in multiple sclerosis. *Multiple Sclerosis Journal*, *24*(10), 1301-1307. https://doi.org/10.1177/1352458518782005

Bittner, S., Oh, J., Havrdová, E. K., Tintoré, M., & Zipp, F. (2021). The potential of serum neurofilament as biomarker for multiple sclerosis. *Brain*, *144*(10), 2954-2963. https://doi.org/10.1093/brain/awab241

Bjornevik, K., Cortese, M., Healy, B. C., Kuhle, J., Mina, M. J., Leng, Y., Elledge, S. J., Niebuhr, D. W., Scher, A. I., & Munger, K. L. (2022). Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*, *375*(6578), 296-301. https://doi.org/ 0.1126/science.abj8222

Bonekamp, N. E., Visseren, F. L., Ruigrok, Y., Cramer, M. J., de Borst, G. J., May, A. M., & Koopal, C. (2023). Leisure-time and occupational physical activity and health outcomes in cardiovascular disease. *Heart*, *109*(9), 686-694. https://doi.org/10.1136/heartjnl-2022-321474

Borgundvaag, E., & Janssen, I. (2017). Objectively measured physical activity and mortality risk among American adults. *American journal of preventive medicine*, *52*(1), e25-e31. https://doi.org/10.1016/j.amepre.2016.09.017

Boziki, M., Theotokis, P., Kesidou, E., Karafoulidou, E., Michailidou, I., Bahar, Y., . . . Grigoriadis, N. (2023). Sex, aging and immunity in multiple sclerosis and experimental autoimmune encephalomyelitis: An intriguing interaction. *Frontiers in Neurology*, *13*, 1104552. https://doi.org/10.3389/fneur.2022.1104552

Braune, S., Bluemich, S., Bruns, C., Dirks, P., Hoffmann, J., Heer, Y., Rouzic, E. M.-L., & Bergmann, A. (2023). The natural history of primary progressive multiple sclerosis: insights from the German NeuroTransData registry. *BMC neurology*, *23*(1), 1-12. https://doi.org/10.1186/s12883-023-03273-9

Bridel, C., Van Wieringen, W. N., Zetterberg, H., Tijms, B. M., Teunissen, C. E., Alvarez-Cermeño, J. C., Andreasson, U., Axelsson, M., Bäckström, D. C., & Bartos, A. (2019). Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA neurology*, *76*(9), 1035-1048. https://doi.org/10.1001/jamaneurol.2019.1534

Briggs, F. B., Gunzler, D. D., Ontaneda, D., & Marrie, R. A. (2017). Smokers with MS have greater decrements in quality of life and disability than non-smokers. *Multiple Sclerosis Journal*, *23*(13), 1772-1781. https://doi.org/10.1177/13524585166851

Briken, S., Rosenkranz, S. C., Keminer, O., Patra, S., Ketels, G., Heesen, C., Hellweg, R., Pless, O., Schulz, K.-H., & Gold, S. M. (2016). Effects of exercise on Irisin, BDNF and IL-6 serum levels in patients with progressive multiple sclerosis. *Journal of neuroimmunology*, *299*, 53-58. https://doi.org/10.1016/j.jneuroim.2016.08.007

Brown, W. M., Davison, G. W., McClean, C. M., & Murphy, M. H. (2015). A systematic review of the acute effects of exercise on immune and inflammatory indices in untrained adults. *Sports medicine-open*, *1*, 1-10. https://doi.org/10.1186/s40798-015-0032-x

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T., MacLean, D., & Pedersen, B. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *The Journal of physiology*, *499*(3), 833-841. https://doi.org/10.1113/jphysiol.1997.sp021972

Buzzard, K., Chan, W. H., Kilpatrick, T., & Murray, S. (2017). Multiple sclerosis: basic and clinical. *Neurodegenerative Diseases: Pathology, Mechanisms, and Potential Therapeutic Targets*, 211-252. https://doi.org/10.1007/978-3-319-57193-5_8

Cabral-Santos, C., Castrillón, C. I., Miranda, R. A., Monteiro, P. A., Inoue, D. S., Campos, E. Z., Hofmann, P., & Lira, F. S. (2016). Inflammatory cytokines and BDNF response to high-intensity intermittent exercise: effect the exercise volume. *Frontiers in Physiology*, *7*, 509. https://doi.org/10.3389/fphys.2016.00509

Caggiula, M., Batocchi, A., Frisullo, G., Angelucci, F., Patanella, A., Sancricca, C., Nociti, V., Tonali, P., & Mirabella, M. (2005). Neurotrophic factors and clinical recovery in relapsing-remitting multiple sclerosis. *Scandinavian journal of immunology*, *62*(2), 176-182. https://doi.org/10.1111/j.1365-3083.2005.01649.x

Cagney, D. N., Sul, J., Huang, R. Y., Ligon, K. L., Wen, P. Y., & Alexander, B. M. (2018). The FDA NIH Biomarkers, EndpointS, and other Tools (BEST) resource in neuro-oncology. *Neuro-oncology*, *20*(9), 1162-1172. https://doi.org/10.1093/neuonc/nox242

Califf, R. M. (2018). Biomarker definitions and their applications. *Experimental Biology and Medicine*, *243*(3), 213-221. https://doi.org/10.1177/1535370217750088

Canning, K. L., & Hicks, A. L. (2020). Benefits of adhering to the Canadian physical activity guidelines for adults with multiple sclerosis beyond aerobic fitness and strength. *International Journal of MS Care*, *22*(1), 15-21. https://doi.org/10.7224/1537-2073.2018-061

Capo, X., Galmes-Panades, A. M., Navas-Enamorado, C., Ortega-Moral, A., Marín, S., Cascante, M., Sánchez-Polo, A., Masmiquel, L., Torrens-Mas, M., & Gonzalez-Freire, M. (2023). Circulating Neurofilament Light Chain Levels Increase with Age and Are Associated with Worse Physical Function and Body Composition in Men but Not in Women. *International Journal of Molecular Sciences*, *24*(16), 12751. https://doi.org/10.3390/ijms241612751

Carnicer-Cáceres, C., Arranz-Amo, J. A., Cea-Arestin, C., Camprodon-Gomez, M., Moreno-Martinez, D., Lucas-Del-Pozo, S., ... & Pintos-Morell, G. (2021). Biomarkers in Fabry disease. Implications for clinical diagnosis and follow-up. *Journal of clinical medicine*, *10*(8), 1664.

Casaletto, K. B., Kornack, J., Paolillo, E. W., Rojas, J. C., VandeBunte, A., Staffaroni, A. S., Lee, S., Heuer, H., Forsberg, L., & Ramos, E. M. (2023). Association of physical activity with neurofilament light chain trajectories in autosomal dominant frontotemporal lobar degeneration variant carriers. *JAMA neurology*, *80*(1), 82-90. https://doi.org/10.1001/jamaneurol.2022.4178

Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public health reports*, *100*(2), 126.

Castellano, V., Patel, D. I., & White, L. J. (2008). Cytokine responses to acute and chronic exercise in multiple sclerosis. *Journal of Applied Physiology*, *104*(6), 1697-1702. https://doi.org/10.1152/japplphysiol.00954.2007

Castellano, V., & White, L. J. (2008). Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. *Journal of the neurological sciences*, *269*(1), 85-91. https://doi.org/10.1016/j.jns.2007.12.030

Chakrapani, A. T. (2021). Biomarkers of Diseases: Their Role in Emergency Medicine. *Neurodegenerative Diseases-Molecular Mechanisms and Current Therapeutic Approaches*.

Chaves, A. R., Kelly, L. P., Moore, C. S., Stefanelli, M., & Ploughman, M. (2019). Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in multiple sclerosis. *Clinical Neurophysiology*, *130*(4), 474-483. https://doi.org/10.1016/j.clinph.2018.12.015

Chinea, A., Ríos-Bedoya, C. F., Vicente, I., Rubí, C., García, G., Rivera, A., Díaz, A., Romero, E. E., Hernández Silvestrini, Y., & Díaz, Y. (2017). Increasing incidence and prevalence of multiple sclerosis in Puerto Rico (2013–2016). *Neuroepidemiology*, *49*(3-4), 106-112. https://doi.org/10.1159/000484090

Chow, L. S., Gerszten, R. E., Taylor, J. M., Pedersen, B. K., Van Praag, H., Trappe, S., Febbraio, M. A., Galis, Z. S., Gao, Y., & Haus, J. M. (2022). Exerkines in health, resilience and disease. *Nature Reviews Endocrinology*, *18*(5), 273-289. https://doi.org/10.1038/s41574-022-00641-2

Choy, E., & Rose-John, S. (2017). Interleukin-6 as a multifunctional regulator: inflammation, immune response, and fibrosis. *Journal of Scleroderma and Related Disorders*, *2*(2_suppl), S1-S5. https://doi.org/10.5301/jsrd.500026

Comabella, M., Sastre-Garriga, J., & Montalban, X. (2016). Precision medicine in multiple sclerosis: biomarkers for diagnosis, prognosis, and treatment response. *Current opinion in neurology*, *29*(3), 254-262. https://doi.org/10.1097/WCO.0000000000000336

Cook, S. D., Dhib-Jalbut, S., Dowling, P., Durelli, L., Ford, C., Giovannoni, G., Halper, J., Harris, C., Herbert, J., & Li, D. (2012). Use of magnetic resonance imaging as well as clinical disease activity in the clinical classification of multiple sclerosis and assessment of its course: a report from an International CMSC Consensus Conference, March 5–7, 2010. *International Journal of MS Care*, *14*(3), 105-114. https://doi.org/10.7224/1537-2073-14.3.105

Correale, J., & Marrodan, M. (2022). Multiple sclerosis and obesity: The role of adipokines. *Frontiers in Immunology*, *13*, 1038393. https://doi.org/10.3389/fimmu.2022.1038393

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., & Sallis, J. F. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine & science in sports & exercise*, *35*(8), 1381-1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Creemers, N., Foster, C., P Porcari, J., L Cress, M., & J de Koning, J. (2017). The physiological mechanism behind the talk test. *Kinesiology*, *49*(1.), 3-8. https://doi.org/10.26582/k.49.1.15

Cruickshank, T., Bartlett, D., Govus, A., Hannan, A., Teo, W. P., Mason, S., Lo, J., & Ziman, M. (2020). The relationship between lifestyle and serum neurofilament light protein in Huntington's disease. *Brain and Behavior*, *10*(5), e01578. https://doi.org/10.1002/brb3.1578

Dalgas, U., Langeskov-Christensen, M., Stenager, E., Riemenschneider, M., & Hvid, L. G. (2019). Exercise as medicine in multiple sclerosis—time for a paradigm shift: preventive, symptomatic, and disease-modifying aspects and perspectives. *Current neurology and neuroscience reports*, *19*, 1-12. https://doi.org/10.1007/s11910-019-1002-3

Dalgas, U., & Stenager, E. (2012). Exercise and disease progression in multiple sclerosis: can exercise slow down the progression of multiple sclerosis? *Therapeutic advances in neurological disorders*, *5*(2), 81-95. https://doi.org/10.1177/1756285611430719

Dalgas, U., Stenager, E., & Ingemann-Hansen, T. (2008). Multiple sclerosis and physical exercise: recommendations for the application of resistance-, endurance-and combined training. *Multiple Sclerosis Journal*, *14*(1), 35-53. https://doi.org/10.1177/1352458507079445

Dalgas, U., Stenager, E., Sloth, M., & Stenager, E. (2015). The effect of exercise on depressive symptoms in multiple sclerosis based on a meta-analysis and critical review of the literature. *European journal of neurology*, *22*(3), 443-e434. https://doi.org/10.1111/ene.12576

Damasceno, A., Dias-Carneiro, R. P. C., Moraes, A. S., Boldrini, V. O., Quintiliano, R. P. S., Farias, A. S., Brandão, C. O., Damasceno, B. P., Dos Santos, L. M. B., & Cendes, F. (2019). Clinical and MRI correlates of CSF neurofilament light chain levels in relapsing and progressive MS. *Multiple sclerosis and related disorders*, *30*, 149-153. https://doi.org/10.1016/j.msard.2019.02.004

Das, S., Dey, M. K., Devireddy, R., & Gartia, M. R. (2023). Biomarkers in Cancer Detection, Diagnosis, and Prognosis. *Sensors*, *24*(1), 37.

data, M. C., & https://mscanada.ca/managing-ms/treatments-for-multiple-sclerosis#toc--disease-modifying-therapies-. *MS Canada data.*

de Jager, W., Bourcier, K., Rijkers, G. T., Prakken, B. J., & Seyfert-Margolis, V. (2009). Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC immunology*, *10*(1), 1-11. https://doi.org/10.1186/1471-2172-10-52

De la Rosa, A., Solana, E., Corpas, R., Bartrés-Faz, D., Pallàs, M., Vina, J., Sanfeliu, C., & Gomez-Cabrera, M. C. (2019). Long-term exercise training improves memory in middle-aged men and modulates peripheral levels of BDNF and Cathepsin B. *Scientific reports*, *9*(1), 3337. https://doi.org/10.1038/s41598-019-40040-8

de Vries, J. E. (1995). Immunosuppressive and anti-inflammatory properties of interleukin 10. *Annals of medicine*, *27*(5), 537-541. https://doi.org/10.3109/07853899509002465

Deckx, N., Wens, I., Nuyts, A. H., Hens, N., De Winter, B. Y., Koppen, G., Goossens, H., Van Damme, P., Berneman, Z. N., & Eijnde, B. O. (2016). 12 weeks of combined endurance and resistance training reduces innate markers of inflammation in a randomized controlled clinical trial in patients with multiple sclerosis. *Mediators of Inflammation*, *2016*. https://doi.org/10.1155/2016/6789276

Deisenhammer, F., Zetterberg, H., Fitzner, B., & Zettl, U. K. (2019). The cerebrospinal fluid in multiple sclerosis. *Frontiers in Immunology*, *10*, 726. https://doi.org/10.3389/fimmu.2019.00726

Delcoigne, B., Manouchehrinia, A., Barro, C., Benkert, P., Michalak, Z., Kappos, L., Leppert, D., Tsai, J. A., Plavina, T., & Kieseier, B. C. (2020). Blood neurofilament light levels segregate treatment effects in multiple sclerosis. *Neurology*, *94*(11), e1201-e1212. https://doi.org/10.1212/WNL.0000000000009097

Desai, P., Dhana, K., DeCarli, C., Wilson, R. S., McAninch, E. A., Evans, D. A., & Rajan, K. B. (2022). Examination of neurofilament light chain serum concentrations, physical activity, and cognitive decline in older adults. *JAMA Network Open*, *5*(3), e223596-e223596. https://doi.org/10.1001/jamanetworkopen.2022.3596

Devasahayam, A. J. J., Kelly, L. P. P., Williams, J. B. B., Moore, C. S., & Ploughman, M. (2021). Fitness shifts the balance of BDNF and IL-6 from inflammation to repair among people with progressive multiple sclerosis. *Biomolecules*, *11*(4), 504. https://doi.org/10.3390/biom11040504

Dhama, K., Latheef, S. K., Dadar, M., Samad, H. A., Munjal, A., Khandia, R., Karthik, K., Tiwari, R., Yatoo, M. I., & Bhatt, P. (2019). Biomarkers in stress related diseases/disorders: diagnostic, prognostic, and therapeutic values. *Frontiers in molecular biosciences*, *6*, 91. https://doi.org/10.3389/fmolb.2019.00091

Diechmann, M. D., Campbell, E., Coulter, E., Paul, L., Dalgas, U., & Hvid, L. G. (2021). Effects of exercise training on neurotrophic factors and subsequent neuroprotection in persons with multiple sclerosis—a systematic review and meta-analysis. *Brain Sciences*, *11*(11), 1499. https://doi.org/10.3390/brainsci11111499

Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest*, *118*(2), 503-508. https://doi.org 10.1378/chest.118.2.503

Dinoff, A., Herrmann, N., Swardfager, W., Liu, C. S., Sherman, C., Chan, S., & Lanctot, K. L. (2016). The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): a meta-analysis. *PloS one*, *11*(9), e0163037. https://doi.org 10.1371/journal.pone.0163037

Diouf, I., Malpas, C. B., Sharmin, S., Roos, I., Horakova, D., Havrdova, E. K., Patti, F., Shaygannejad, V., Ozakbas, S., & Eichau, S. (2023). Effectiveness of multiple disease-modifying therapies in relapsing-remitting multiple sclerosis: causal inference to emulate a multiarm randomised trial. *Journal of Neurology, Neurosurgery & Psychiatry*, *94*(12), 1004-1011. https://doi.org/0.1136/jnnp-2023-331499

Disanto, G., Adiutori, R., Dobson, R., Martinelli, V., Dalla Costa, G., Runia, T., Evdoshenko, E., Thouvenot, E., Trojano, M., & Norgren, N. (2016). Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. *Journal of Neurology, Neurosurgery & Psychiatry*, *87*(2), 126-129. https://doi.org/10.1136/jnnp-2014-309690

Disanto, G., Barro, C., Benkert, P., Naegelin, Y., Schädelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., & Leppert, D. (2017). Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. *Annals of neurology*, *81*(6), 857-870. https://doi.org/10.1002/ana.24954

Disanto, G., Zecca, C., MacLachlan, S., Sacco, R., Handunnetthi, L., Meier, U. C., Simpson, A., McDonald, L., Rossi, A., & Benkert, P. (2018). Prodromal symptoms of multiple sclerosis in primary care. *Annals of neurology*, *83*(6), 1162-1173. https://doi.org/10.1002/ana.25247

Divo, M. J., Martinez, C. H., & Mannino, D. M. (2014). Ageing and the epidemiology of multimorbidity. *European Respiratory Journal*, *44*(4), 1055-1068. https://doi.org/0.1183/09031936.00059814

Eckert, A., Karen, S., Beck, J., Brand, S., Hemmeter, U., Hatzinger, M., & Holsboer-Trachsler, E. (2017). The link between sleep, stress and BDNF. *European Psychiatry*, *41*(S1), S282-S282. https://doi.org/10.1016/j.eurpsy.2017.02.132

Eldadah, B. A. (2010). Fatigue and fatigability in older adults. *PM&R*, *2*(5), 406-413. https://doi.org/10.1016/j.pmrj.2010.03.022

Elkhalii-Wilhelm, S., Sippel, A., Riemann-Lorenz, K., Kofahl, C., Scheiderbauer, J., Arnade, S., Kleiter, I., Schmidt, S., & Heesen, C. (2022). Experiences of persons with Multiple Sclerosis with lifestyle adjustment–A qualitative interview study. *Plos one*, *17*(5), e0268988. https://doi.org/10.1371/journal.pone.0268988

Ensari, I., Motl, R. W., & Pilutti, L. A. (2014). Exercise training improves depressive symptoms in people with multiple sclerosis: results of a meta-analysis. *Journal of psychosomatic research*, *76*(6), 465-471. https://doi.org/10.1016/j.jpsychores.2014.03.014

Enzinger, C., & Fazekas, F. (2015). Measuring gray matter and white matter damage in MS: why this is not enough. *Frontiers in Neurology*, *6*, 56. https://doi.org/10.3389/fneur.2015.00056

Ercan, Z., Bilek, F., & Demir, C. F. (2021). The effect of aerobic exercise on Neurofilament light chain and glial Fibrillary acidic protein level in patients with relapsing remitting type multiple sclerosis. *Multiple sclerosis and related disorders*, *55*, 103219. https://doi.org/10.1016/j.msard.2021.103219

Ershler, W. B. (1993). Interleukin-6: a cytokine for gerontolgists. *Journal of the American geriatrics society*, *41*(2), 176-181. https://doi.org/10.1111/j.1532-5415.1993.tb02054.x

Ershler, W. B., & Keller, E. T. (2000). Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annual review of medicine*, *51*(1), 245-270. https://doi.org/10.1146/annurev.med.51.1.245

Faramarzi, M., Banitalebi, E., Raisi, Z., Samieyan, M., Saberi, Z., Ghahfarrokhi, M. M., Negaresh, R., & Motl, R. W. (2020). Effect of combined exercise training on pentraxins and pro-inflammatory cytokines in people with multiple sclerosis as a function of disability status. *Cytokine*, *134*, 155196. https://doi.org/10.1016/j.cyto.2020.155196

FDA-NIH Biomarker Working Group. (2016). *BEST (Biomarkers, EndpointS, and other Tools) Resource*. Food and Drug Administration (US). http://www.ncbi.nlm.nih.gov/books/NBK326791/

Ferreira-Atuesta, C., Reyes, S., Giovanonni, G., & Gnanapavan, S. (2021). The evolution of neurofilament light chain in multiple sclerosis. *Frontiers in Neuroscience*, *15*, 642384. https://doi.org/10.3389/fnins.2021.642384

Ferris, L. T., Williams, J. S., & Shen, C.-L. (2007). The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Medicine & science in sports & exercise*, *39*(4), 728-734. https://doi.org/10.1249/mss.0b013e31802f04c7

Florindo, M. (2014). Inflammatory cytokines and physical activity in multiple sclerosis. *International Scholarly Research Notices*, *2014*. https://doi.org/10.1155/2014/151572

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M. P., Invidia, L., Celani, L., & Scurti, M. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of ageing and development*, *128*(1), 92-105. https://doi.org/10.1016/j.mad.2006.11.016

Frederiksen, K. S., Jensen, C. S., Høgh, P., Gergelyffy, R., Waldemar, G., Andersen, B. B., Gottrup, H., Vestergaard, K., Wermuth, L., & Søndergaard, H. B. (2023). Aerobic exercise does not affect serum neurofilament light in patients with mild Alzheimer's disease. *Frontiers in Neuroscience*, *17*, 1108191. https://doi.org/10.3389/fnins.2023.1108191

Freedman, M. S., Gnanapavan, S., Booth, R. A., Calabresi, P. A., Khalil, M., Kuhle, J., . . . Olsson, T. (2024). Guidance for use of neurofilament light chain as a cerebrospinal fluid and blood biomarker in multiple sclerosis management. *Ebiomedicine*, *101*. https://doi.org/10.1016/j.ebiom.2024.104970

Fujinami, A., Ohta, K., Obayashi, H., Fukui, M., Hasegawa, G., Nakamura, N., Kozai, H., Imai, S., & Ohta, M. (2008). Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: relationship to glucose metabolism and biomarkers of insulin resistance. *Clinical biochemistry*, *41*(10-11), 812-817. https://doi.org/10.1016/j.clinbiochem.2008.03.003

Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis research & therapy*, *8*(2), 1-6. https://doi.org/10.1186/ar1917

Gaetani, L., Blennow, K., Calabresi, P., Di Filippo, M., Parnetti, L., & Zetterberg, H. (2019). Neurofilament light chain as a biomarker in neurological disorders. *Journal of Neurology, Neurosurgery & Psychiatry*, *90*(8), 870-881. https://doi.org/10.1136/jnnp-2018-320106

Gajofatto, A., & Benedetti, M. D. (2015). Treatment strategies for multiple sclerosis: when to start, when to change, when to stop? *World Journal of Clinical Cases: WJCC*, *3*(7), 545. https://doi.org/10.12998/wjcc.v3.i7.545

Garbers, C., Heink, S., Korn, T., & Rose-John, S. (2018). Interleukin-6: designing specific therapeutics for a complex cytokine. *Nature reviews Drug discovery*, *17*(6), 395-412. https://doi.org/10.1038/nrd.2018.45

García-Gutiérrez, M. S., Navarrete, F., Sala, F., Gasparyan, A., Austrich-Olivares, A., & Manzanares, J. (2020). Biomarkers in psychiatry: concept, definition, types and relevance to the clinical reality. *Frontiers in psychiatry*, *11*, 432. https://doi.org/10.3389/fpsyt.2020.00432

Geng, T. C., & Mark, V. W. (2015). Clinical neurorestorative progress in multiple sclerosis. *Journal of Neurorestoratology*, *3*(1), 83-90. https://doi.org/10.2147/JN.S74146

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: a guide for non-statisticians. *International journal of endocrinology and metabolism*, *10*(2), 486. https://doi.org/ 0.5812/ijem.3505

Ghasemi, N., Razavi, S., & Nikzad, E. (2017). Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. *Cell Journal (Yakhteh)*, *19*(1), 1. https://doi.org/10.22074/cellj.2016.4867

Giesser, B. S. (2015). Exercise in the management of persons with multiple sclerosis. *Therapeutic advances in neurological disorders*, *8*(3), 123-130. https://doi.org/10.1177/1756285615576663

Gnanapavan, S., & Giovannoni, G. (2015). Developing biomarkers for MS. *Emerging and Evolving Topics in Multiple Sclerosis Pathogenesis and Treatments*, 179-194. https://doi.org/10.1007/7854_2014_362

Gold, S. M., Schulz, K.-H., Hartmann, S., Mladek, M., Lang, U. E., Hellweg, R., Reer, R., Braumann, K.-M., & Heesen, C. (2003). Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *Journal of neuroimmunology*, *138*(1-2), 99-105. https://doi.org/10.1016/s0165-5728(03)00121-8

Goldhammer, E., Tanchilevitch, A., Maor, I., Beniamini, Y., Rosenschein, U., & Sagiv, M. (2005). Exercise training modulates cytokines activity in coronary heart disease patients. *International journal of cardiology*, *100*(1), 93-99. https://doi.org/10.1016/j.ijcard.2004.08.073

Golzari, Z., Shabkhiz, F., Soudi, S., Kordi, M. R., & Hashemi, S. M. (2010). Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. *International immunopharmacology*, *10*(11), 1415-1419. https://doi.org/10.1016/j.intimp.2010.08.008

Gravesteijn, A. S., Beckerman, H., Willemse, E. A., Hulst, H. E., de Jong, B. A., Teunissen, C. E., & de Groot, V. (2023). Brain-derived neurotrophic factor, neurofilament light and glial fibrillary acidic protein do not change in response to aerobic training in people with MS-related fatigue–a secondary analysis of a randomized controlled trial. *Multiple sclerosis and related disorders*, *70*, 104489. https://doi.org/10.1016/j.msard.2022.104489

Grzegorski, T., & Losy, J. (2020). What do we currently know about the clinically isolated syndrome suggestive of multiple sclerosis? An update. *Reviews in the Neurosciences*, *31*(3), 335-349. https://doi.org/10.1515/revneuro-2019-0084

Guan, Y., Jakimovski, D., Ramanathan, M., Weinstock-Guttman, B., & Zivadinov, R. (2019). The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to in vivo imaging. *Neural regeneration research*, *14*(3), 373. https://doi.org/10.4103/1673-5374.245462

Gunnarsson, M., Malmeström, C., Axelsson, M., Sundström, P., Dahle, C., Vrethem, M., Olsson, T., Piehl, F., Norgren, N., & Rosengren, L. (2011). Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. *Annals of neurology*, *69*(1), 83-89. https://doi.org/10.1002/ana.22247

Haines, J. D., Inglese, M., & Casaccia, P. (2011). Axonal damage in multiple sclerosis. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, *78*(2), 231-243. https://doi.org/10.1002/msj.20246

Halabchi, F., Alizadeh, Z., Sahraian, M. A., & Abolhasani, M. (2017). Exercise prescription for patients with multiple sclerosis; potential benefits and practical recommendations. *BMC neurology*, *17*(1), 1-11. https://doi.org/10.1186/s12883-017-0960-9

Hartwig, F. P., Borges, M. C., Horta, B. L., Bowden, J., & Davey Smith, G. (2017). Inflammatory Biomarkers and Risk of Schizophrenia: A 2-Sample Mendelian Randomization Study. *JAMA Psychiatry*, *74*(12), 1226–1233. https://doi.org/10.1001/jamapsychiatry.2017.3191

Haskell, W. L., Lee, I.-M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., Macera, C. A., Heath, G. W., Thompson, P. D., & Bauman, A. (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*, *116*(9), 1081. https://doi.org/10.1249/mss.0b013e3180616b27

Hauser, S. L., & Cree, B. A. (2020). Treatment of multiple sclerosis: a review. *The American journal of medicine*, *133*(12), 1380-1390. e1382. https://doi.org/10.1016/j.amjmed.2020.05.049

Hayashi, M., Mistunaga, F., Ohira, K., & Shimizu, K. (2001). Changes in BDNF-immunoreactive structures in the hippocampal formation of the aged macaque monkey. *Brain research*, *918*(1-2), 191-196. https://doi.org/10.1016/s0006-8993(01)03002-5

He, A., Merkel, B., Brown, J. W. L., Ryerson, L. Z., Kister, I., Malpas, C. B., Sharmin, S., Horakova, D., Havrdova, E. K., & Spelman, T. (2020). Timing of high-efficacy therapy for multiple sclerosis: a retrospective observational cohort study. *The Lancet Neurology*, *19*(4), 307-316. https://doi.org/10.1016/S1474-4422(20)30067-3

Healy, B. C., Ali, E. N., Guttmann, C. R., Chitnis, T., Glanz, B. I., Buckle, G., Houtchens, M., Stazzone, L., Moodie, J., & Berger, A. M. (2009). Smoking and disease progression in multiple sclerosis. *Archives of neurology*, *66*(7), 858-864. https://doi.org/10.1001/archneurol.2009.122

Hedström, A. K. (2023). Risk factors for multiple sclerosis in the context of Epstein-Barr virus infection. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1212676

Heilbronn, L. K., & Campbell, L. V. (2008). Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Current pharmaceutical design*, *14*(12), 1225-1230. https://doi.org/10.2174/138161208784246153

Heine, M., van de Port, I., Rietberg, M. B., van Wegen, E. E., & Kwakkel, G. (2015). Exercise therapy for fatigue in multiple sclerosis. *Cochrane database of systematic reviews*(9). https://doi.org/10.1002/14651858.CD009956.pub2

Heine, M., Wens, I., Langeskov-Christensen, M., Verschuren, O., Eijnde, B. O., Kwakkel, G., & Dalgas, U. (2016). Cardiopulmonary fitness is related to disease severity in multiple sclerosis. *Multiple Sclerosis Journal*, *22*(2), 231-238. https://doi.org/10.1177/1352458515581437

Hermesdorf, M., Leppert, D., Maceski, A., Benkert, P., Wellmann, J., Wiendl, H., Kuhle, J., & Berger, K. (2022). Longitudinal analyses of serum neurofilament light and associations with obesity indices and bioelectrical impedance parameters. *Scientific reports*, *12*(1), 15863. https://doi.org/10.1038/s41598-022-20398-y

Hernán, M. A., Jick, S. S., Logroscino, G., Olek, M. J., Ascherio, A., & Jick, H. (2005). Cigarette smoking and the progression of multiple sclerosis. *Brain*, *128*(6), 1461-1465. https://doi.org/10.1093/brain/awh471

Hirano, T. (2021). IL-6 in inflammation, autoimmunity and cancer. *International immunology*, *33*(3), 127-148. https://doi.org/10.1093/intimm/dxaa078

Hockings, P., Saeed, N., Simms, R., Smith, N., Hall, M. G., Waterton, J. C., & Sourbron, S. (2020). MRI biomarkers. In *Advances in magnetic resonance technology and applications* (Vol. 1, pp. liii-lxxxvi). Elsevier. https://doi.org/10.1016/B978-0-12-817057-1.00002-0

Høglund, R. A., & Maghazachi, A. A. (2014). Multiple sclerosis and the role of immune cells. *World journal of experimental medicine*, *4*(3), 27. https://doi.org/10.5493/wjem.v4.i3.27

Hosang, G. M., Shiles, C., Tansey, K. E., McGuffin, P., & Uher, R. (2014). Interaction between stress and the BDNFVal66Met polymorphism in depression: a systematic review and meta-analysis. *BMC medicine*, *12*(1), 1-11. https://doi.org/10.1186/1741-7015-12-7

Huff, T., Tadi, P., Weisbrod, L. J., & Varacallo, M. (2017). Neuroanatomy, cerebrospinal fluid.

Hunter, C. A., & Jones, S. A. (2015). IL-6 as a keystone cytokine in health and disease. *Nature immunology*, *16*(5), 448-457. Bookshelf ID: NBK470578

Huynh, T. L., Silveira, S. L., Jeng, B., & Motl, R. W. (2022). Association of disease outcomes with physical activity in multiple sclerosis: A cross-sectional study. *Rehabilitation Psychology*, *67*(3), 421. https://doi.org/10.1037/rep0000454

Iannetta, D., Keir, D. A., Fontana, F. Y., Inglis, E. C., Mattu, A. T., Paterson, D. H., Pogliaghi, S., & Murias, J. M. (2021). Evaluating the accuracy of using fixed ranges of METs to categorize exertional intensity in a heterogeneous group of healthy individuals: implications for cardiorespiratory fitness and health outcomes. *Sports medicine*, *51*(11), 2411-2421. https://doi.org/10.1007/s40279-021-01476-z

Jakimovski, D., Guan, Y., Ramanathan, M., Weinstock-Guttman, B., & Zivadinov, R. (2019). Lifestyle-based modifiable risk factors in multiple sclerosis: review of experimental and clinical findings. *Neurodegenerative disease management*, *9*(3), 149-172. https://doi.org/10.2217/nmt-2018-0046

Jensen, S. K., Michaels, N. J., Ilyntskyy, S., Keough, M. B., Kovalchuk, O., & Yong, V. W. (2018). Multimodal enhancement of remyelination by exercise with a pivotal role for oligodendroglial PGC1α. *Cell reports*, *24*(12), 3167-3179. https://doi.org/10.1016/j.celrep.2018.08.060

Jeon, Y. K., & Ha, C. H. (2017). The effect of exercise intensity on brain derived neurotrophic factor and memory in adolescents. *Environmental health and preventive medicine*, *22*(1), 1-6. https://doi.org/10.1186/s12199-017-0643-6

Jetté, M., Sidney, K., & Blümchen, G. (1990). Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical cardiology*, *13*(8), 555-565. https://doi.org/10.1002/clc.4960130809

Joisten, N., Rademacher, A., Warnke, C., Proschinger, S., Schenk, A., Walzik, D., Knoop, A., Thevis, M., Steffen, F., & Bittner, S. (2021). Exercise diminishes plasma neurofilament light chain and reroutes the kynurenine pathway in multiple sclerosis. *Neurology-Neuroimmunology Neuroinflammation*, *8*(3). https://doi.org/10.1212/NXI.0000000000000982

Kalb, R., Brown, T. R., Coote, S., Costello, K., Dalgas, U., Garmon, E., Giesser, B., Halper, J., Karpatkin, H., & Keller, J. (2020). Exercise and lifestyle physical activity recommendations for people with multiple

sclerosis throughout the disease course. *Multiple Sclerosis Journal*, *26*(12), 1459-1469. https://doi.org/10.1177/1352458520915629

Kallaur, A. P., Oliveira, S. R., Delicato de Almeida, E. R., Kaminami Morimoto, H., Lopes, J., de Carvalho Jennings Pereira, W. L., Marques Andrade, R., Muliterno Pelegrino, L., Donizete Borelli, S., & Kaimen-Maciel, D. R. (2013). Cytokine profile in relapsing-remitting multiple sclerosis patients and the association between progression and activity of the disease. *Molecular Medicine Reports*, *7*(3), 1010-1020. https://doi.org/10.3892/mmr.2013.1256

Kapoor, R., Smith, K. E., Allegretta, M., Arnold, D. L., Carroll, W., Comabella, M., Furlan, R., Harp, C., Kuhle, J., & Leppert, D. (2020). Serum neurofilament light as a biomarker in progressive multiple sclerosis. *Neurology*, *95*(10), 436-444. https://doi.org/10.1212/WNL.0000000000010346

Katoh-Semba, R., Semba, R., Takeuchi, I. K., & Kato, K. (1998). Age-related changes in levels of brain-derived neurotrophic factor in selected brain regions of rats, normal mice and senescence-accelerated mice: a comparison to those of nerve growth factor and neurotrophin-3. *Neuroscience research*, *31*(3), 227-234. https://doi.org/10.1016/s0168-0102(98)00040-6

Katsavos, S., & Anagnostouli, M. (2013). Biomarkers in multiple sclerosis: an up-to-date overview. *Multiple sclerosis international*, *2013*. https://doi.org/10.1155/2013/340508

Kelly, L. P., Devasahayam, A. J., Chaves, A. R., Wallack, E. M., McCarthy, J., Basset, F. A., & Ploughman, M. (2017). Intensifying functional task practice to meet aerobic training guidelines in stroke survivors. *Frontiers in Physiology*, *8*, 809. https://doi.org/10.3389/fphys.2017.00809

Khaibullin, T., Ivanova, V., Martynova, E., Cherepnev, G., Khabirov, F., Granatov, E., Rizvanov, A., & Khaiboullina, S. (2017). Elevated levels of proinflammatory cytokines in cerebrospinal fluid of multiple sclerosis patients. *Frontiers in Immunology*, *8*, 531. https://doi.org/10.3389/fimmu.2017.00531

Khalil, M., Pirpamer, L., Hofer, E., Voortman, M. M., Barro, C., Leppert, D., Benkert, P., Ropele, S., Enzinger, C., & Fazekas, F. (2020). Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nature communications*, *11*(1), 812. https://doi.org/10.1038/s41467-020-14612-6

Kierkegaard, M., Lundberg, I. E., Olsson, T., Johansson, S., Ygberg, S., Opava, C., Holmqvist, L. W., & Piehl, F. (2016). High-intensity resistance training in multiple sclerosis—An exploratory study of effects on immune markers in blood and cerebrospinal fluid, and on mood, fatigue, health-related quality of life, muscle strength, walking and cognition. *Journal of the neurological sciences*, *362*, 251-257. https://doi.org/10.1016/j.jns.2016.01.063

King, M., Kelly, L. P., Wallack, E. M., Hasan, S. M., Kirkland, M. C., Curtis, M. E., Chatterjee, T., McCarthy, J., & Ploughman, M. (2019). Serum levels of insulin-like growth factor-1 and brain-derived neurotrophic factor as potential recovery biomarkers in stroke. *Neurological Research*, *41*(4), 354-363. https://doi.org/10.1080/01616412.2018.1564451

Kinnett-Hopkins, D., Adamson, B., Rougeau, K., & Motl, R. (2017). People with MS are less physically active than healthy controls but as active as those with other chronic diseases: an updated meta-

analysis. *Multiple sclerosis and related disorders*, *13*, 38-43. https://doi.org/10.1016/j.msard.2017.01.016

Kjølhede, T., Vissing, K., & Dalgas, U. (2012). Multiple sclerosis and progressive resistance training: a systematic review. *Multiple Sclerosis Journal*, *18*(9), 1215-1228. https://doi.org/10.1177/135245851243741

Klaren, R. E., Hubbard, E. A., Motl, R. W., Pilutti, L. A., Wetter, N. C., & Sutton, B. P. (2015). Objectively measured physical activity is associated with brain volumetric measurements in multiple sclerosis. *Behavioural Neurology*, *2015*. https://doi.org/10.1155/2015/482536

Klineova, S., & Lublin, F. D. (2018). Clinical course of multiple sclerosis. *Cold Spring Harbor perspectives in medicine*, *8*(9), a028928. https://doi.org/10.1101/cshperspect.a028928

Knaepen, K., Goekint, M., Heyman, E. M., & Meeusen, R. (2010). Neuroplasticity—exercise-induced response of peripheral brain-derived neurotrophic factor. *Sports medicine*, *40*(9), 765-801. https://doi.org/10.2165/11534530-000000000-00000

Knudsen, J. G., Gudiksen, A., Bertholdt, L., Overby, P., Villesen, I., Schwartz, C. L., & Pilegaard, H. (2017). Skeletal muscle IL-6 regulates muscle substrate utilization and adipose tissue metabolism during recovery from an acute bout of exercise. *PloS one*, *12*(12), e0189301. https://doi.org/10.1371/journal.pone.0189301

Kohn, C. G., Coleman, C. I., Michael White, C., Sidovar, M. F., & Sobieraj, D. M. (2014). Mobility, walking and physical activity in persons with multiple sclerosis. *Current Medical Research and Opinion*, *30*(9), 1857-1862. https://doi.org/10.1185/03007995.2014.921147

Kołtuniuk, A., & Chojdak-Łukasiewicz, J. (2022). Adherence to Therapy in Patients with Multiple Sclerosis. *International Journal of Environmental Research and Public Health*, *19*(4), 2203. https://doi.org/10.3390/ijerph19042203

Krabbe, K., Nielsen, A., Krogh-Madsen, R., Plomgaard, P., Rasmussen, P., Erikstrup, C., Fischer, C., Lindegaard, B., Petersen, A., & Taudorf, S. (2007). Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia*, *50*, 431-438. https://doi.org/10.1007/s00125-006-0537-4

Kuhle, J., Barro, C., Andreasson, U., Derfuss, T., Lindberg, R., Sandelius, Å., Liman, V., Norgren, N., Blennow, K., & Zetterberg, H. (2016). Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *54*(10), 1655-1661. https://doi.org/10.1515/cclm-2015-1195

Kuhle, J., Disanto, G., Lorscheider, J., Stites, T., Chen, Y., Dahlke, F., Francis, G., Shrinivasan, A., Radue, E.-W., & Giovannoni, G. (2015). Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. *Neurology*, *84*(16), 1639-1643. https://doi.org/10.1212/WNL.000000000000149

Kuhle, J., Kropshofer, H., Haering, D. A., Kundu, U., Meinert, R., Barro, C., Dahlke, F., Tomic, D., Leppert, D., & Kappos, L. (2019). Blood neurofilament light chain as a biomarker of MS disease activity and

treatment response. *Neurology*, *92*(10), e1007-e1015. https://doi.org/10.1212/WNL.00000000000070

Kujala, U. M., Vaara, J. P., Kainulainen, H., Vasankari, T., Vaara, E., & Kyröläinen, H. (2019). Associations of aerobic fitness and maximal muscular strength with metabolites in young men. *JAMA Network Open*, *2*(8), e198265-e198265. https://doi.org/10.1001/jamanetworkopen.2019.8265

Kupjetz, M., Joisten, N., Rademacher, A., Gonzenbach, R., Bansi, J., & Zimmer, P. (2023). Cycling in primary progressive multiple sclerosis (CYPRO): study protocol for a randomized controlled superiority trial evaluating the effects of high-intensity interval training in persons with primary progressive multiple sclerosis. *BMC neurology*, *23*(1), 162. https://doi.org/10.1186/s12883-023-03187-6

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, *33*(11), 1444-1444. https://doi.org/10.1212/wnl.33.11.1444

Ladang, A., Kovacs, S., Lengelé, L., Locquet, M., Reginster, J.-Y., Bruyère, O., & Cavalier, E. (2022). Neurofilament light chain concentration in an aging population. *Aging Clinical and Experimental Research*, *34*(2), 331-339. https://doi.org/10.1007/s40520-021-02054-z

Langer-Gould, A., Brara, S. M., Beaber, B. E., & Zhang, J. L. (2013). Incidence of multiple sclerosis in multiple racial and ethnic groups. *Neurology*, *80*(19), 1734-1739. https://doi.org/10.1212/WNL.0b013e3182918cc2

Langeskov-Christensen, M., Bisson, E. J., Finlayson, M. L., & Dalgas, U. (2017). Potential pathophysiological pathways that can explain the positive effects of exercise on fatigue in multiple sclerosis: a scoping review. *Journal of the neurological sciences*, *373*, 307-320. https://doi.org/10.1016/j.jns.2017.01.002

Langeskov-Christensen, M., Heine, M., Kwakkel, G., & Dalgas, U. (2015). Aerobic capacity in persons with multiple sclerosis: a systematic review and meta-analysis. *Sports medicine*, *45*, 905-923. https://doi.org/10.1007/s40279-015-0307-x

Langeskov-Christensen, M., Hvid, L. G., Nygaard, M. K. E., Ringgaard, S., Jensen, H. B., Nielsen, H. H., Petersen, T., Stenager, E., Eskildsen, S. F., & Dalgas, U. (2021). Efficacy of high-intensity aerobic exercise on brain MRI measures in multiple sclerosis. *Neurology*, *96*(2), e203-e213. https://doi.org/10.1212/WNL.0000000000011241

Lassmann, H. (2018). Multiple sclerosis pathology. *Cold Spring Harbor perspectives in medicine*, *8*(3). https://doi.org/10.1101/cshperspect.a028936

Laterza, O. F., Hendrickson, R. C., & Wagner, J. A. (2007). Molecular biomarkers. *Drug Information Journal*, *41*(5), 573-585. https://doi.org/10.1177/009286150704100

Lee, J. D., Guimond, C., Yee, I. M., Vilariño-Güell, C., Wu, Z.-Y., Traboulsee, A. L., & Sadovnick, A. D. (2015). Incidence of multiple sclerosis and related disorders in Asian populations of British Columbia. *Canadian Journal of Neurological Sciences*, *42*(4), 235-241. https://doi.org/10.1017/cjn.2015.36

Li, B., Lang, N., & Cheng, Z.-F. (2016). Serum levels of brain-derived neurotrophic factor are associated with diabetes risk, complications, and obesity: a cohort study from Chinese patients with type 2 diabetes. *Molecular neurobiology*, *53*, 5492-5499. https://doi.org/10.1007/s12035-015-9461-2

Lie, I. A., Weeda, M. M., Mattiesing, R. M., Mol, M. A., Pouwels, P. J., Barkhof, F., Torkildsen, Ø., Bø, L., Myhr, K.-M., & Vrenken, H. (2022). Relationship between white matter lesions and gray matter atrophy in multiple sclerosis: A systematic review. *Neurology*, *98*(15), e1562-e1573. https://doi.org/10.1212/WNL.0000000000200006

Liu, P., & Nusslock, R. (2018). Exercise-mediated neurogenesis in the hippocampus via BDNF. Front Neurosci. 2018; 12: 52. In: Epub 2018/02/23. https://doi.org/10.3389/fnins.2018.00052

Liu, Y., Lu, L., Yang, H., Wu, X., Luo, X., Shen, J., Xiao, Z., Zhao, Y., Du, F., & Chen, Y. (2023). Dysregulation of immunity by cigarette smoking promotes inflammation and cancer: A review. *Environmental Pollution*, 122730. https://doi.org/10.1016/j.envpol.2023.122730

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., & Virchow, J. C. (2005). The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiology of aging*, *26*(1), 115-123. https://doi.org/10.1016/j.neurobiolaging.2004.03.002

Loonstra, F. C., de Ruiter, L. R., Schoonheim, M. M., Moraal, B., Strijbis, E. M., de Jong, B. A., & Uitdehaag, B. M. (2023). The role of diet in multiple sclerosis onset and course: results from a nationwide retrospective birth-year cohort. *Annals of clinical and translational neurology*, *10*(8), 1268-1283. https://doi.org/10.1002/acn3.51788

Loonstra, F. C., de Ruiter, L. R., Strijbis, E. M., de Jong, B. A., & Uitdehaag, B. M. (2023). The association between weight during early life and multiple sclerosis onset in a nationwide Dutch birth year cohort. *Nutritional neuroscience*, 1-7. https://doi.org/10.1080/1028415X.2023.2225271

Lozinski, B. M., & Yong, V. W. (2022). Exercise and the brain in multiple sclerosis. *Multiple Sclerosis Journal*, *28*(8), 1167-1172. https://doi.org/10.1177/1352458520969099

Macdonald, E., Buchan, D., Cerexhe, L., Renfrew, L., & Sculthorpe, N. (2022). Accelerometer measured physical activity and sedentary time in individuals with Multiple Sclerosis versus age matched controls: A systematic review and meta-analysis. *Multiple sclerosis and related disorders*, 104462. https://doi.org/10.1016/j.msard.2022.104462

MacIntosh, B. R., Murias, J. M., Keir, D. A., & Weir, J. M. (2021). What is moderate to vigorous exercise intensity? *Frontiers in physiology*, 1481. https://doi.org/10.3389/fphys.2021.682233

Madsen, L. T., Dalgas, U., Hvid, L. G., & Bansi, J. (2019). A cross-sectional study on the relationship between cardiorespiratory fitness, disease severity and walking speed in persons with multiple sclerosis. *Multiple sclerosis and related disorders*, *29*, 35-40. https://doi.org/10.1016/j.msard.2019.01.024

Magyari, M., & Sorensen, P. S. (2020). Comorbidity in multiple sclerosis. *Frontiers in Neurology*, *11*, 851. https://doi.org/10.3389/fneur.2020.00851

Majdinasab, N., Motl, R. W., Mokhtarzade, M., Zimmer, P., Ranjbar, R., Keytsman, C., Cullen, T., Negaresh, R., & Baker, J. S. (2018). Acute responses of cytokines and adipokines to aerobic exercise in relapsing vs. remitting women with multiple sclerosis. *Complementary therapies in clinical practice*, *31*, 295-301. https://doi.org/10.1016/j.ctcp.2018.03.004

Majkić-Singh, N. (2011). What is a biomarker?: From its discovery to clinical application. *Journal of Medical Biochemistry*, *30*(3), 186-192. https://doi.org/10.2478/v10011-011-0029-z

Makhani, N., & Tremlett, H. (2021). The multiple sclerosis prodrome. *Nature Reviews Neurology*, *17*(8), 515-521. https://doi.org/10.1038/s41582-021-00519-3

Manouchehrinia, A., Piehl, F., Hillert, J., Kuhle, J., Alfredsson, L., Olsson, T., & Kockum, I. (2020). Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Annals of clinical and translational neurology*, *7*(1), 139-143. https://doi.org/10.1002/acn3.50972

Manouchehrinia, A., Tench, C. R., Maxted, J., Bibani, R. H., Britton, J., & Constantinescu, C. S. (2013). Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. *Brain*, *136*(7), 2298-2304. https://doi.org/10.1093/brain/awt139

Marrie, R., Rudick, R., Horwitz, R., Cutter, G., Tyry, T., Campagnolo, D., & Vollmer, T. (2010). Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *Neurology*, *74*(13), 1041-1047. https://doi.org/10.1212/WNL.0b013e3181d6b125

Martin, S.-J., McGlasson, S., Hunt, D., & Overell, J. (2019). Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case–control studies. *Journal of Neurology, Neurosurgery & Psychiatry*, *90*(9), 1059-1067. https://doi.org/10.1136/jnnp-2018-319190

Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K., & Initiative, A. s. D. N. (2017). Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA neurology*, *74*(5), 557-566. https://doi.org/10.1001/jamaneurol.2016.6117

Matute-Blanch, C., Villar, L. M., Álvarez-Cermeño, J. C., Rejdak, K., Evdoshenko, E., Makshakov, G., Nazarov, V., Lapin, S., Midaglia, L., & Vidal-Jordana, A. (2018). Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. *Brain*, *141*(4), 1085-1093. https://doi.org/10.1093/brain/awy021

McEvoy, J. W., Nasir, K., DeFilippis, A. P., Lima, J. A., Bluemke, D. A., Hundley, W. G., Barr, R. G., Budoff, M. J., Szklo, M., & Navas-Acien, A. (2015). Relationship of cigarette smoking with inflammation and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*, *35*(4), 1002-1010. https://doi.org/10.1161/ATVBAHA.114.304960

Medicine, A. C. o. S. (2013). *ACSM's guidelines for exercise testing and prescription*. Lippincott williams & wilkins.

Mee-Inta, O., Zhao, Z.-W., & Kuo, Y.-M. (2019). Physical exercise inhibits inflammation and microglial activation. *Cells*, *8*(7), 691. https://doi.org/10.3390/cells8070691

Mey, G. M., Mahajan, K. R., & DeSilva, T. M. (2023). Neurodegeneration in multiple sclerosis. *WIREs Mechanisms of Disease*, *15*(1), e1583. https://doi.org/10.1002/wsbm.1583

Meyer-Arndt, L., Kerkering, J., Kuehl, T., Infante, A. G., Paul, F., Rosiewicz, K. S., Siffrin, V., & Alisch, M. (2023). Inflammatory cytokines associated with multiple sclerosis directly induce alterations of neuronal cytoarchitecture in human neurons. *Journal of Neuroimmune Pharmacology*, 1-15. https://doi.org/10.1007/s11481-023-10059-w

Miller, D. H., & Leary, S. M. (2007). Primary-progressive multiple sclerosis. *The Lancet Neurology*, *6*(10), 903-912. https://doi.org/ 10.1016/S1474-4422(07)70243-0

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. *Frontiers in cellular neuroscience*, 363. https://doi.org/10.3389/fncel.2019.00363

Mokhtarzade, M., Motl, R., Negaresh, R., Zimmer, P., Khodadoost, M., Baker, J. S., Patel, D., Majdinasab, N., & Ranjbar, R. (2018). Exercise-induced changes in neurotrophic factors and markers of blood-brain barrier permeability are moderated by weight status in multiple sclerosis. *Neuropeptides*, *70*, 93-100. https://doi.org/10.1016/j.npep.2018.05.010

Mokhtarzade, M., Ranjbar, R., Majdinasab, N., Patel, D., & Molanouri Shamsi, M. (2017). Effect of aerobic interval training on serum IL-10, TNFα, and adipokines levels in women with multiple sclerosis: possible relations with fatigue and quality of life. *Endocrine*, *57*, 262-271. https://doi.org/10.1007/s12020-017-1337-y

Moosaie, F., Mohammadi, S., Saghazadeh, A., Dehghani Firouzabadi, F., & Rezaei, N. (2023). Brain-derived neurotrophic factor in diabetes mellitus: A systematic review and meta-analysis. *PloS one*, *18*(2), e0268816. https://doi.org/10.1371/journal.pone.0268816

Motl, R. (2021). Multiple Sclerosis Minute: Physical Activity & MS.

Motl, R. W., Arnett, P., Smith, M., Barwick, F., Ahlstrom, B., & Stover, E. (2008). Worsening of symptoms is associated with lower physical activity levels in individuals with multiple sclerosis. *Multiple Sclerosis Journal*, *14*(1), 140-142. https://doi.org/10.1177/1352458507079126

Motl, R. W., & Baird, J. F. (2021). Cardiorespiratory fitness and moderate-to-vigorous physical activity in older adults with multiple sclerosis. *Multiple Sclerosis Journal–Experimental, Translational and Clinical*, *7*(4), 20552173211057514. https://doi.org/10.1177/20552173211057514

Motl, R. W., Fernhall, B., McAuley, E., & Cutter, G. (2011). Physical activity and self-reported cardiovascular comorbidities in persons with multiple sclerosis: evidence from a cross-sectional analysis. *Neuroepidemiology*, *36*(3), 183-191. https://doi.org/10.1159/000327749

Motl, R. W., Gappmaier, E., Nelson, K., & Benedict, R. H. (2011). Physical activity and cognitive function in multiple sclerosis. *Journal of Sport and Exercise Psychology*, *33*(5), 734-741. https://doi.org/10.1123/jsep.33.5.734

Motl, R. W., & Gosney, J. (2008). Effect of exercise training on quality of life in multiple sclerosis: a meta-analysis. *Multiple Sclerosis Journal*, *14*(1), 129-135. https://doi.org/10.1177/1352458507080464

Motl, R. W., McAuley, E., & Snook, E. M. (2005). Physical activity and multiple sclerosis: a meta-analysis. *Multiple Sclerosis Journal*, *11*(4), 459-463. https://doi.org/0.1191/1352458505ms1188oa

Motl, R. W., McAuley, E., Snook, E. M., & Gliottoni, R. C. (2009). Physical activity and quality of life in multiple sclerosis: intermediary roles of disability, fatigue, mood, pain, self-efficacy and social support. *Psychology, health & medicine*, *14*(1), 111-124. https://doi.org/10.1080/13548500802241902

Motl, R. W., & Pilutti, L. A. (2016). Is physical exercise a multiple sclerosis disease modifying treatment? *Expert review of neurotherapeutics*, *16*(8), 951-960. https://doi.org/10.1080/14737175.2016.1193008

Motl, R. W., Pilutti, L. A., Hubbard, E. A., Wetter, N. C., Sosnoff, J. J., & Sutton, B. P. (2015). Cardiorespiratory fitness and its association with thalamic, hippocampal, and basal ganglia volumes in multiple sclerosis. *NeuroImage: Clinical*, *7*, 661-666. https://doi.org/10.1016/j.nicl.2015.02.017

Motl, R. W., & Sandroff, B. M. (2018). Exercise as a countermeasure to declining central nervous system function in multiple sclerosis. *Clinical therapeutics*, *40*(1), 16-25. https://doi.org/10.1016/j.clinthera.2017.12.001

Motl, R. W., Sandroff, B. M., & Benedict, R. H. (2022). Moderate-to-vigorous physical activity is associated with processing speed, but not learning and memory, in cognitively impaired persons with multiple sclerosis. *Multiple sclerosis and related disorders*, *63*, 103833. https://doi.org/10.1016/j.msard.2022.103833

Motl, R. W., Sandroff, B. M., Kwakkel, G., Dalgas, U., Feinstein, A., Heesen, C., Feys, P., & Thompson, A. J. (2017). Exercise in patients with multiple sclerosis. *The Lancet Neurology*, *16*(10), 848-856. https://doi.org/10.1016/S1474-4422(17)30281-8

Motl, R. W., Snook, E. M., & Schapiro, R. T. (2008). Symptoms and physical activity behavior in individuals with multiple sclerosis. *Research in nursing & health*, *31*(5), 466-475. https://doi.org/10.1002/nur.20274

Motl, R. W., Snook, E. M., Wynn, D. R., & Vollmer, T. (2008). Physical activity correlates with neurological impairment and disability in multiple sclerosis. *The Journal of nervous and mental disease*, *196*(6), 492-495. https://doi.org/10.1097/NMD.0b013e318177351b

Motl, R. W., Suh, Y., & Weikert, M. (2010). Symptom cluster and quality of life in multiple sclerosis. *Journal of pain and symptom management*, *39*(6), 1025-1032. https://doi.org/10.1016/j.jpainsymman.2009.11.312

Mughal, M. R., Baharani, A., Chigurupati, S., Son, T. G., Chen, E., Yang, P., ... & Mattson, M. P. (2011). Electroconvulsive shock ameliorates disease processes and extends survival in huntingtin mutant mice. *Human molecular genetics*, *20*(4), 659-669.

Mulero, P., Maroto-Izquierdo, S., Redondo, N., Gonzalo-Benito, H., Chavarría-Miranda, A., Calvo, H., Cabero, M. I., Hernandez, M., Nieto, M. L., & Tellez, N. (2023). Effect of resistance exercise training on plasma neurofilaments in multiple sclerosis: a proof of concept for future designs. *Neurological Sciences*, 1-4. https://doi.org/10.1007/s10072-023-06896-5

Muñoz-Cánoves, P., Scheele, C., Pedersen, B. K., & Serrano, A. L. (2013). Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *The FEBS journal*, *280*(17), 4131-4148. https://doi.org/10.1111/febs.12338

Neal, W. N., Cederberg, K. L., Jeng, B., Sasaki, J. E., & Motl, R. W. (2020). Is symptomatic fatigue associated with physical activity and sedentary behaviors among persons with multiple sclerosis? *Neurorehabilitation and neural repair*, *34*(6), 505-511. https://doi.org/10.1177/1545968320916159

Negaresh, R., Motl, R., Zimmer, P., Mokhtarzade, M., & Baker, J. (2019). Effects of exercise training on multiple sclerosis biomarkers of central nervous system and disease status: a systematic review of intervention studies. *European journal of neurology*, *26*(5), 711-721. https://doi.org/10.1111/ene.13929

Nelson, M. E., Rejeski, W. J., Blair, S. N., Duncan, P. W., Judge, J. O., King, A. C., Macera, C. A., & Castaneda-Sceppa, C. (2007). Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. *Circulation*, *116*(9), 1094. https://doi.org/10.1249/mss.0b013e3180616aa2

Neurath, M. F., & Finotto, S. (2011). IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine & growth factor reviews*, *22*(2), 83-89. https://doi.org/10.1016/j.cytogfr.2011.02.003. Epub 2011 Mar 5

Ng, A. V., & Kent-Braun, J. A. (1997). Quantitation of lower physical activity in persons with multiple sclerosis. *Medicine and Science in Sports and Exercise*, *29*(4), 517-523. https://doi.org/10.1097/00005768-199704000-00014

Ng, T. K. S., Ho, C. S. H., Tam, W. W. S., Kua, E. H., & Ho, R. C. M. (2019). Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): a systematic review and meta-analysis. *International journal of molecular sciences*, *20*(2), 257.

Nicholson, M., Yoo, S., Craig, G. A., Murray, S. S., & Fletcher, J. L. (2021). BDNF-TrkB Signaling in Lifelong Central Nervous System Myelination and Myelin Repair. In *Handbook of Neurotoxicity* (pp. 1-28). Springer. https://doi.org/10.1007/978-3-030-71519-9_219-1

Nieman, D. C., Nehlsen-Cannarella, S. L., Fagoaga, O. R., Henson, D., Utter, A., Davis, J. M., Williams, F., & Butterworth, D. E. (1998). Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Medicine and Science in Sports and Exercise*, *30*(5), 671-678. https://doi.org/10.1097/00005768-199805000-00005

Ning, L., & Wang, B. (2022). Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: A systematic review and meta-analysis. *Plos one*, *17*(9), e0274565. https://doi.org/10.1371/journal.pone.0274565

Nociti, V., Romozzi, M., & Mirabella, M. (2022). Update on multiple sclerosis molecular biomarkers to monitor treatment effects. *Journal of Personalized Medicine*, *12*(4), 549. https://doi.org/10.3390/jpm12040549

Northoff, H., & Berg, A. (1991). Immunologic mediators as parameters of the reaction to strenuous exercise. *International journal of sports medicine*, *12*(S 1), S9-S15. https://doi.org/ 10.1055/s-2007-1024743

Novakova, L., Zetterberg, H., Sundström, P., Axelsson, M., Khademi, M., Gunnarsson, M., Malmeström, C., Svenningsson, A., Olsson, T., & Piehl, F. (2017). Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology*, *89*(22), 2230-2237. https://doi.org/10.1212/WNL.0000000000004683

Okazaki, S., Hishimoto, A., Otsuka, I., Watanabe, Y., Numata, S., Boku, S., Shimmyo, N., Kinoshita, M., Inoue, E., Ohmori, T., Someya, T., & Sora, I. (2018). Increased serum levels and promoter polymorphisms of macrophage migration inhibitory factor in schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *83*, 33–41. https://doi.org/10.1016/j.pnpbp.2018.01.001

Opal, S. M., & DePalo, V. A. (2000). Anti-inflammatory cytokines. *Chest*, *117*(4), 1162-1172. https://doi.org/10.1378/chest.117.4.1162

Ozkul, C., Guclu-Gunduz, A., Irkec, C., Fidan, I., Aydin, Y., Ozkan, T., & Yazici, G. (2018). Effect of combined exercise training on serum brain-derived neurotrophic factor, suppressors of cytokine signaling 1 and 3 in patients with multiple sclerosis. *Journal of neuroimmunology*, *316*, 121-129. https://doi.org/10.1016/j.jneuroim.2018.01.002

Öztuna, D., Elhan, A. H., & Tüccar, E. (2006). Investigation of four different normality tests in terms of type 1 error rate and power under different distributions. *Turkish Journal of Medical Sciences*, *36*(3), 171-176.

Pala, P., Hussell, T., & Openshaw, P. J. (2000). Flow cytometric measurement of intracellular cytokines. *Journal of immunological methods*, *243*(1-2), 107-124. https://doi.org/10.1016/s0022-1759(00)00230-1

Palle, P., Monaghan, K. L., Milne, S. M., & Wan, E. C. (2017). Cytokine signaling in multiple sclerosis and its therapeutic applications. *Medical sciences*, *5*(4), 23. https://doi.org/10.3390/medsci5040023

Papathanassoglou, E. D., Miltiadous, P., & Karanikola, M. N. (2015). May BDNF be implicated in the exercise-mediated regulation of inflammation? critical review and synthesis of evidence. *Biological Research for Nursing*, *17*(5), 521-539. https://doi.org/10.1177/1099800414555411

Paul, A., Comabella, M., & Gandhi, R. (2019). Biomarkers in multiple sclerosis. *Cold Spring Harbor perspectives in medicine*, *9*(3), a029058. https://doi.org/10.1101/cshperspect.a029058

Pedersen, B. (2007). IL-6 signalling in exercise and disease. *Biochemical Society Transactions*, *35*(5), 1295-1297. https://doi.org/10.1042/BST0351295

Pedersen, B. K., & Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological reviews*, *88*(4), 1379-1406. https://doi.org/10.1152/physrev.90100.2007

Péter, M., Török, W., Petrovics-Balog, A., Vígh, L., Vécsei, L., & Balogh, G. (2020). Cerebrospinal fluid lipidomic biomarker signatures of demyelination for multiple sclerosis and Guillain–Barré syndrome. *Scientific reports*, *10*(1), 18380. https://doi.org/10.1038/s41598-020-75502-x

Petersen, A. M. W., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, *98*(4), 1154-1162. https://doi.org/10.1152/japplphysiol.00164.2004

Petersen, E. R., Oturai, A. B., Koch-Henriksen, N., Magyari, M., Sørensen, P. S., Sellebjerg, F., & Søndergaard, H. B. (2018). Smoking affects the interferon beta treatment response in multiple sclerosis. *Neurology*, *90*(7), e593-e600. https://doi.org/10.1212/WNL.0000000000004949

Piercy, K. L., Troiano, R. P., Ballard, R. M., Carlson, S. A., Fulton, J. E., Galuska, D. A., George, S. M., & Olson, R. D. (2018). The physical activity guidelines for Americans. *Jama*, *320*(19), 2020-2028. https://doi.org/10.1001/jama.2018.14854

Pilutti, L. A., Greenlee, T. A., Motl, R. W., Nickrent, M. S., & Petruzzello, S. J. (2013). Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosomatic medicine*, *75*(6), 575-580. https://doi.org/10.1097/PSY.0b013e31829b4525

Pilutti, L. A., Platta, M. E., Motl, R. W., & Latimer-Cheung, A. E. (2014). The safety of exercise training in multiple sclerosis: a systematic review. *Journal of the neurological sciences*, *343*(1-2), 3-7. https://doi.org/10.1016/j.jns.2014.05.016

Platta, M. E., Ensari, I., Motl, R. W., & Pilutti, L. A. (2016). Effect of exercise training on fitness in multiple sclerosis: a meta-analysis. *Archives of physical medicine and rehabilitation*, *97*(9), 1564-1572. https://doi.org/10.1016/j.apmr.2016.01.023

Ploughman, M., Yong, V. W., Spermon, B., Goelz, S., & Giovannoni, G. (2022). Remyelination trial failures: Repercussions of ignoring neurorehabilitation and exercise in repair. *Multiple sclerosis and related disorders*, *58*, 103539. https://doi.org/10.1016/j.msard.2022.103539

Ponnappan, S., & Ponnappan, U. (2011). Aging and immune function: molecular mechanisms to interventions. https://doi.org/10.1089/ars.2010.3228

Poorolajal, J., Bahrami, M., Karami, M., & Hooshmand, E. (2017). Effect of smoking on multiple sclerosis: a meta-analysis. *Journal of public health*, *39*(2), 312-320. https://doi.org/10.1093/pubmed/fdw030

Porter, L., Shoushtarizadeh, A., Jelinek, G. A., Brown, C. R., Lim, C. K., de Livera, A. M., Jacobs, K. R., & Weiland, T. J. (2020). Metabolomic biomarkers of multiple sclerosis: a systematic review. *Frontiers in molecular biosciences*, *7*, 574133. https://doi.org/10.3389/fmolb.2020.574133

Prakash, R. S., Snook, E. M., Motl, R. W., & Kramer, A. F. (2010). Aerobic fitness is associated with gray matter volume and white matter integrity in multiple sclerosis. *Brain research*, *1341*, 41-51. https://doi.org/10.1016/j.brainres.2009.06.063

Price, R. D., Milne, S. A., Sharkey, J., & Matsuoka, N. (2007). Advances in small molecules promoting neurotrophic function. *Pharmacology & therapeutics*, *115*(2), 292-306. https://doi.org/10.1016/j.pharmthera.2007.03.005

Proschinger, S., Kuhwand, P., Rademacher, A., Walzik, D., Warnke, C., Zimmer, P., & Joisten, N. (2022). Fitness, physical activity, and exercise in multiple sclerosis: a systematic review on current evidence for interactions with disease activity and progression. *Journal of Neurology*, *269*(6), 2922-2940. https://doi.org/10.1007/s00415-021-10935-6

Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., Skalska, A., Jonas, M., Franek, E., & Mossakowska, M. (2016). Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immunity & Ageing*, *13*, 1-12. https://doi.org/10.1186/s12979-016-0076-x

Raffin, J., Rolland, Y., Aggarwal, G., Nguyen, A. D., Morley, J. E., Li, Y., Bateman, R. J., Vellas, B., & Barreto, P. d. S. (2021). Associations between physical activity, blood-based biomarkers of neurodegeneration, and cognition in healthy older adults: the MAPT study. *The Journals of Gerontology: Series A*, *76*(8), 1382-1390. https://doi.org/10.1093/gerona/glab094

Ramani, T., Auletta, C. S., Weinstock, D., Mounho-Zamora, B., Ryan, P. C., Salcedo, T. W., & Bannish, G. (2015). Cytokines: the good, the bad, and the deadly. *International journal of toxicology*, *34*(4), 355-365. https://doi.org/10.1177/1091581815584918

Ranadive, S. M., Yan, H., Weikert, M., Lane, A. D., Linden, M. A., Baynard, T., Motl, R. W., & Fernhall, B. (2012). Vascular dysfunction and physical activity in multiple sclerosis. *Medicine & science in sports & exercise*, *44*(2), 238-243. https://doi.org/10.1249/MSS.0b013e31822d7997

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V., Leick, L., Hart, E., Secher, N. H., Pedersen, B. K., & Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental physiology*, *94*(10), 1062-1069. https://doi.org/10.1113/expphysiol.2009.048512

Rea, I. M., Gibson, D. S., McGilligan, V., McNerlan, S. E., Alexander, H. D., & Ross, O. A. (2018). Age and age-related diseases: role of inflammation triggers and cytokines. *Frontiers in Immunology*, 586. https://doi.org/10.3389/fimmu.2018.00586

Reed, J. L., & Pipe, A. L. (2014). The talk test: a useful tool for prescribing and monitoring exercise intensity. *Current opinion in cardiology*, *29*(5), 475-480. https://doi.org/10.1097/HCO.0000000000000097

Reiner, M., Niermann, C., Jekauc, D., & Woll, A. (2013). Long-term health benefits of physical activity--a systematic review of longitudinal studies. *BMC Public Health*, *13*, 813. https://doi.org/10.1186/1471-2458-13-813

Reyes, S., Smets, I., Holden, D., Carrillo-Loza, K., Christmas, T., Bianchi, L., Ammoscato, F., Turner, B., Marta, M., & Schmierer, K. (2020). CSF neurofilament light chain testing as an aid to determine treatment strategies in MS. *Neurology-Neuroimmunology Neuroinflammation*, *7*(6). https://doi.org/10.1212/NXI.0000000000000880

Robb, M. A., McInnes, P. M., & Califf, R. M. (2016). Biomarkers and surrogate endpoints: developing common terminology and definitions. *Jama*, *315*(11), 1107-1108. https://doi.org/10.1001/jama.2016.2240

Robson-Ansley, P., Barwood, M., Canavan, J., Hack, S., Eglin, C., Davey, S., Hewitt, J., Hull, J., & Ansley, L. (2009). The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest. *Cytokine*, *45*(2), 111-116. https://doi.org/10.1016/j.cyto.2008.11.006

Romme Christensen, J., Komori, M., von Essen, M. R., Ratzer, R., Börnsen, L., Bielekova, B., & Sellebjerg, F. (2019). CSF inflammatory biomarkers responsive to treatment in progressive multiple sclerosis capture residual inflammation associated with axonal damage. *Multiple Sclerosis Journal*, *25*(7), 937-946. https://doi.org/10.1177/1352458518774880

Roohi, E., Jaafari, N., & Hashemian, F. (2021). On inflammatory hypothesis of depression: what is the role of IL-6 in the middle of the chaos? *Journal of neuroinflammation*, *18*(1), 1-15. https://doi.org/10.1186/s12974-021-02100-7

Rooney, S., Riemenschneider, M., Dalgas, U., Jørgensen, M.-L. K., Michelsen, A.-S., Brønd, J. C., & Hvid, L. G. (2021). Physical activity is associated with neuromuscular and physical function in patients with multiple sclerosis independent of disease severity. *Disability and rehabilitation*, *43*(5), 632-639. https://doi.org/10.1080/09638288.2019.1634768

Roshanisefat, H., Bahmanyar, S., Hillert, J., Olsson, T., & Montgomery, S. (2014). Multiple sclerosis clinical course and cardiovascular disease risk–S wedish cohort study. *European journal of neurology*, *21*(11), 1353-e1388. https://doi.org/10.1111/ene.12518

Sabatino Jr, J. J., Pröbstel, A.-K., & Zamvil, S. S. (2019). B cells in autoimmune and neurodegenerative central nervous system diseases. *Nature Reviews Neuroscience*, *20*(12), 728-745. https://doi.org/10.1038/s41583-019-0233-2

Sadovnick, D. (2019). The place of environmental factors in multiple sclerosis: genes, environment and the interactions thereof in the etiology of multiple sclerosis. *Revue neurologique*, *175*(10), 593-596. https://doi.org/10.1016/j.neurol.2019.08.003

Salzer, J., Svenningsson, A., & Sundström, P. (2010). Neurofilament light as a prognostic marker in multiple sclerosis. *Multiple Sclerosis Journal*, *16*(3), 287–292. https://doi.org/10.1177/1352458509359725

Sandrini, L., Di Minno, A., Amadio, P., Ieraci, A., Tremoli, E., & Barbieri, S. S. (2018). Association between obesity and circulating brain-derived neurotrophic factor (BDNF) levels: Systematic review of literature and meta-analysis. *International Journal of Molecular Sciences*, *19*(8), 2281. https://doi.org/10.3390/ijms19082281

Sandroff, B., Dlugonski, D., Weikert, M., Suh, Y., Balantrapu, S., & Motl, R. (2012). Physical activity and multiple sclerosis: new insights regarding inactivity. *Acta Neurologica Scandinavica*, *126*(4), 256-262. https://doi.org/10.1111/j.1600-0404.2011.01634.x

Sarchielli, P., Greco, L., Stipa, A., Floridi, A., & Gallai, V. (2002). Brain-derived neurotrophic factor in patients with multiple sclerosis. *Journal of neuroimmunology*, *132*(1-2), 180-188. https://doi.org/10.1016/S0165-5728(02)00319-3

Savšek, L., Stergar, T., Strojnik, V., Alojz, I., Koren, A., Špiclin, Ž., & Jazbec, S. Š. (2021). Impact of aerobic exercise on clinical and magnetic resonance imaging biomarkers in persons with multiple sclerosis: An exploratory randomized controlled trial. *Journal of Rehabilitation Medicine*, *53*(4). https://doi.org/10.2340/16501977-2814

Scalfari, A., Neuhaus, A., Daumer, M., Ebers, G., & Muraro, P. (2011). Age and disability accumulation in multiple sclerosis. *Neurology*, *77*(13), 1246-1252. https://doi.org/10.1212/WNL.0b013e318230a17d

Scalzo, P., Kümmer, A., Bretas, T. L., Cardoso, F., & Teixeira, A. L. (2010). Serum levels of brain-derived neurotrophic factor correlate with motor impairment in Parkinson's disease. *Journal of neurology*, *257*, 540-545.

Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1813*(5), 878-888. https://doi.org/10.1016/j.bbamcr.2011.01.034

Scherr, J., Wolfarth, B., Christle, J. W., Pressler, A., Wagenpfeil, S., & Halle, M. (2013). Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *European journal of applied physiology*, *113*, 147-155. https://doi.org/10.1007/s00421-012-2421-x

Schmalhofer, M.-L., Markus, M. R., Gras, J. C., Kopp, J., Janowitz, D., Grabe, H.-J., Groß, S., Ewert, R., Gläser, S., & Albrecht, D. (2019). Sex-Specific associations of brain-derived neurotrophic factor and cardiorespiratory fitness in the general population. *Biomolecules*, *9*(10), 630. https://doi.org/10.3390/biom9100630

Schreiner, T.-G., & Genes, T.-M. (2021). Obesity and multiple sclerosis—A multifaceted association. *Journal of Clinical Medicine*, *10*(12), 2689. https://doi.org/10.3390/jcm10122689

Schulz, K.-H., Gold, S. M., Witte, J., Bartsch, K., Lang, U. E., Hellweg, R., Reer, R., Braumann, K.-M., & Heesen, C. (2004). Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. *Journal of the neurological sciences*, *225*(1-2), 11-18. https://doi.org/10.1016/j.jns.2004.06.009

Sebastião, E., Pilutti, L. A., & Motl, R. W. (2019). Aerobic fitness and instrumental activities of daily living in people with multiple sclerosis: a cross-sectional study. *International Journal of MS Care*, *21*(1), 23-28. https://doi.org/10.7224/1537-2073.2017-078

Shahim, P., Gren, M., Liman, V., Andreasson, U., Norgren, N., Tegner, Y., ... & Blennow, K. (2016). Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. *Scientific reports*, *6*(1), 36791.

Shephard, R. J. (1988). PAR-Q, Canadian Home Fitness Test and exercise screening alternatives. *Sports medicine*, *5*, 185-195.

Shobeiri, P., Karimi, A., Momtazmanesh, S., Teixeira, A. L., Teunissen, C. E., van Wegen, E. E., Hirsch, M. A., Yekaninejad, M. S., & Rezaei, N. (2022). Exercise-induced increase in blood-based brain-derived neurotrophic factor (BDNF) in people with multiple sclerosis: A systematic review and meta-analysis of exercise intervention trials. *PloS one*, *17*(3), e0264557. https://doi.org/10.1371/journal.pone.0264557

Shobeiri, P., Seyedmirzaei, H., Karimi, N., Rashidi, F., Teixeira, A. L., Brand, S., Sadeghi-Bahmani, D., & Rezaei, N. (2022). IL-6 and TNF-α responses to acute and regular exercise in adult individuals with multiple sclerosis (MS): a systematic review and meta-analysis. *European Journal of Medical Research*, *27*(1), 185. https://doi.org/10.1186/s40001-022-00814-9

Silveira, S. L., Cederberg, K. L., Jeng, B., Sikes, E. M., Sandroff, B. M., Jones, C. D., & Motl, R. W. (2021). Do physical activity and social cognitive theory variable scores differ across symptom cluster severity groups in multiple sclerosis? *Disability and Health Journal*, *14*(4), 101163. https://doi.org/10.1016/j.dhjo.2021.101163

Simpson, R. J., Hammacher, A., Smith, D. K., Matthews, J. M., & Ward, L. D. (1997). Interleukin-6: Structure-function relationships. *Protein science*, *6*(5), 929-955. https://doi.org/10.1002/pro.5560060501

Snook, E. M., Motl, R. W., & Gliottoni, R. C. (2009). The effect of walking mobility on the measurement of physical activity using accelerometry in multiple sclerosis. *Clinical Rehabilitation*, *23*(3), 248-258. https://doi.org/10.1177/0269215508101757

Sohrabji, F., & Lewis, D. K. (2006). Estrogen–BDNF interactions: implications for neurodegenerative diseases. *Frontiers in neuroendocrinology*, *27*(4), 404-414.

Sokratous, D., Charalambous, C. C., Papanicolaou, E. Z., Michailidou, K., & Konstantinou, N. (2023). Investigation of in-phase bilateral exercise effects on corticospinal plasticity in relapsing remitting multiple sclerosis: A registered report single-case concurrent multiple baseline design across five subjects. *PloS one*, *18*(3), e0272114. https://doi.org/10.1371/journal.pone.0272114

Soldan, S. S., & Lieberman, P. M. (2023). Epstein–Barr virus and multiple sclerosis. *Nature Reviews Microbiology*, *21*(1), 51-64. https://doi.org/10.1038/s41579-022-00770-5

Sprenger, H., Jacobs, C., Nain, M., Gressner, A., Prinz, H., Wesemann, W., & Gemsa, D. (1992). Enhanced release of cytokines, interleukin-2 receptors, and neopterin after long-distance running. *Clinical*

immunology and immunopathology, *63*(2), 188-195. https://doi.org/10.1016/0090-1229(92)90012-d

Spring, S., Food, & Administration, D. (2016). Bethesda (MD); National Institutes of Health (US). *BEST (Biomarkers, EndpointS, and Other Tools) Resource*.

Stampanoni Bassi, M., Iezzi, E., Drulovic, J., Pekmezovic, T., Gilio, L., Furlan, R., Finardi, A., Marfia, G. A., Sica, F., & Centonze, D. (2020). IL-6 in the cerebrospinal fluid signals disease activity in multiple sclerosis. *Frontiers in cellular neuroscience*, *14*, 120. https://doi.org/10.3389/fncel.2020.00120

Starkie, R., Ostrowski, S. R., Jauffred, S., Febbraio, M., & Pedersen, B. K. (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. *The FASEB Journal*, *17*(8), 1-10. https://doi.org/10.1096/fj.02-0670fje

Steensberg, A., Fischer, C. P., Keller, C., Møller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *285*(2), E433-E437. https://doi.org/10.1152/ajpendo.00074.2003

Steensberg, A., Toft, A. D., Bruunsgaard, H., Sandmand, M., Halkjær-Kristensen, J., & Pedersen, B. K. (2001). Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *Journal of Applied Physiology*, *91*(4), 1708-1712. https://doi.org/10.1152/jappl.2001.91.4.1708

Stelmasiak, Z., Kozioł-Montewka, M., Dobosz, B., Rejdak, K., Bartosik-Psujek, H., Mitosek-Szewczyk, K., & Belniak-Legieć, E. (2000). Interleukin-6 concentration in serum and cerebrospinal fluid in multiple sclerosis patients. *Medical science monitor: international medical journal of experimental and clinical research*, *6*(6), 1104-1108. Med Sci Monit 2000; 6(6): CR1104-1108 :: ID: 507608

Strath, S. J., Kaminsky, L. A., Ainsworth, B. E., Ekelund, U., Freedson, P. S., Gary, R. A., Richardson, C. R., Smith, D. T., & Swartz, A. M. (2013). Guide to the assessment of physical activity: clinical and research applications: a scientific statement from the American Heart Association. *Circulation*, *128*(20), 2259-2279. https://doi.org/10.1161/01.cir.0000435708.67487.da

Stroud, N. M., & Minahan, C. L. (2009). The impact of regular physical activity on fatigue, depression and quality of life in persons with multiple sclerosis. *Health and quality of life outcomes*, *7*, 1-10. https://doi.org/10.1186/1477-7525-7-68

Suh, Y., Motl, R. W., & Mohr, D. C. (2010). Physical activity, disability, and mood in the early stage of multiple sclerosis. *Disability and Health Journal*, *3*(2), 93-98. https://doi.org/10.1016/j.dhjo.2009.09.002

Svensson, M., Lexell, J., & Deierborg, T. (2015). Effects of physical exercise on neuroinflammation, neuroplasticity, neurodegeneration, and behavior: what we can learn from animal models in clinical settings. *Neurorehabilitation and neural repair*, *29*(6), 577-589. https://doi.org/10.1177/1545968314562108

Świderek-Matysiak, M., Oset, M., Domowicz, M., Galazka, G., Namiecińska, M., & Stasiołek, M. (2023). Cerebrospinal Fluid Biomarkers in Differential Diagnosis of Multiple Sclerosis and Systemic

Inflammatory Diseases with Central Nervous System Involvement. *Biomedicines*, *11*(2), 425. https://doi.org/10.3390/biomedicines11020425

Szuhany, K. L., Bugatti, M., & Otto, M. W. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *Journal of psychiatric research*, *60*, 56-64. https://doi.org/10.1016/j.jpsychires.2014.10.003

Tadayon Zadeh, F., Amini, H., Habibi, S., Shahedi, V., Isanejad, A., & Akbarpour, M. (2021). The effects of 8-week combined exercise training on inflammatory markers in women with multiple sclerosis. *Neurodegenerative Diseases*, *20*(5-6), 212-216. https://doi.org/10.1159/000518580

Tafti, D., Ehsan, M., & Xixis, K. (2022). Multiple Sclerosis. StatPearls. Treasure Island (FL). In: StatPearls Publishing Copyright.

Tallner, A., Waschbisch, A., Wenny, I., Schwab, S., Hentschke, C., Pfeifer, K., & Mäurer, M. (2012). Multiple sclerosis relapses are not associated with exercise. *Multiple Sclerosis Journal*, *18*(2), 232-235. https://doi.org/10.1177/1352458511415143

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor perspectives in biology*, *6*(10), a016295. https://doi.org/10.1101/cshperspect.a016295

Tavakoli-Ardakani, M., Mehrpooya, M., Mehdizadeh, M., Hajifathali, A., & Abdolahi, A. (2015). Association between Interlukin-6 (IL-6), Interlukin-10 (IL-10) and depression in patients undergoing Hematopoietic stem cell transplantation. *International Journal of Hematology-Oncology and Stem Cell Research*, *9*(2), 80.

Telano, L. N., & Baker, S. (2018). Physiology, cerebral spinal fluid. Bookshelf ID: NBK519007

Teunissen, C., Koel-Simmelink, M., Pham, T., Knol, J., Khalil, M., Trentini, A., Killestein, J., Nielsen, J., Vrenken, H., & Popescu, V. (2011). Identification of biomarkers for diagnosis and progression of MS by MALDI-TOF mass spectrometry. *Multiple Sclerosis Journal*, *17*(7), 838-850. https://doi.org/10.1177/1352458511399614

Thebault, S., Abdoli, M., Fereshtehnejad, S.-M., Tessier, D., Tabard-Cossa, V., & Freedman, M. S. (2020). Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. *Scientific reports*, *10*(1), 10381. https://doi.org/10.1038/s41598-020-67504-6

Thebault, S., Booth, R. A., & Freedman, M. S. (2020). Blood neurofilament light chain: the neurologist's troponin? *Biomedicines*, *8*(11), 523. https://doi.org/10.3390/biomedicines8110523

Thebault, S., Booth, R. A., Rush, C. A., MacLean, H., & Freedman, M. S. (2021). Serum neurofilament light chain measurement in MS: hurdles to clinical translation. *Frontiers in Neuroscience*, *15*, 654942. https://doi.org/10.3389/fnins.2021.654942

Thebault, S., Reaume, M., Marrie, R. A., Marriott, J. J., Furlan, R., Laroni, A., Booth, R. A., Uccelli, A., & Freedman, M. S. (2022). High or increasing serum NfL is predictive of impending multiple sclerosis relapses. *Multiple sclerosis and related disorders*, *59*, 103535. https://doi.org/10.1016/j.msard.2022.103535

Thomas, S., Reading, J., & Shephard, R. J. (1992). Revision of the physical activity readiness questionnaire (PAR-Q). *Canadian journal of sport sciences*.

Thompson, A. J., Banwell, B. L., Barkhof, F., Carroll, W. M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., & Freedman, M. S. (2018). Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology*, *17*(2), 162-173. https://doi.org/10.1016/S1474-4422(17)30470-2

Thompson, A. J., Baranzini, S. E., Geurts, J., Hemmer, B., & Ciccarelli, O. (2018). Multiple sclerosis. *The Lancet*, *391*(10130), 1622-1636. https://doi.org/10.1016/S0140-6736(18)30481-1

Thompson, D. K., Huffman, K. M., Kraus, W. E., & Kraus, V. B. (2012). Critical appraisal of four IL-6 immunoassays. *PloS one*, *7*(2), e30659. https://doi.org/10.1371/journal.pone.0030659

Thormann, A., Sørensen, P. S., Koch-Henriksen, N., Laursen, B., & Magyari, M. (2017). Comorbidity in multiple sclerosis is associated with diagnostic delays and increased mortality. *Neurology*, *89*(16), 1668-1675. https://doi.org/10.1212/WNL.0000000000004508

Tomassini, V., & Palace, J. (2009). Multiple sclerosis lesions: insights from imaging techniques. *Expert review of neurotherapeutics*, *9*(9), 1341-1359. https://doi.org/10.1586/ern.09.83

Tremblay, M. S., Colley, R. C., Saunders, T. J., Healy, G. N., & Owen, N. (2010). Physiological and health implications of a sedentary lifestyle. *Applied Physiology, Nutrition, and Metabolism*, *35*(6), 725-740. https://doi.org/10.1139/H10-079

Tremlett, H., Munger, K. L., & Makhani, N. (2022). The multiple sclerosis prodrome: evidence to action. *Frontiers in Neurology*, *12*, 761408. https://doi.org/10.3389/fneur.2021.761408

Tudor-Locke, C. E., & Myers, A. M. (2001). Challenges and opportunities for measuring physical activity in sedentary adults. *Sports medicine*, *31*, 91-100. https://doi.org/10.2165/00007256-200131020-00002

Tur, C., Carbonell-Mirabent, P., Cobo-Calvo, Á., Otero-Romero, S., Arrambide, G., Midaglia, L., Castilló, J., Vidal-Jordana, Á., Rodríguez-Acevedo, B., & Zabalza, A. (2023). Association of early progression independent of relapse activity with long-term disability after a first demyelinating event in multiple sclerosis. *JAMA neurology*, *80*(2), 151-160. https://doi.org/10.1001/jamaneurol.2022.4655

Tutuncu, M., Tang, J., Zeid, N. A., Kale, N., Crusan, D. J., Atkinson, E. J., Siva, A., Pittock, S. J., Pirko, I., & Keegan, B. M. (2013). Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. *Multiple Sclerosis Journal*, *19*(2), 188-198. https://doi.org/10.1177/1352458512451510

Uphaus, T., Steffen, F., Muthuraman, M., Ripfel, N., Fleischer, V., Groppa, S., Ruck, T., Meuth, S. G., Pul, R., & Kleinschnitz, C. (2021). NfL predicts relapse-free progression in a longitudinal multiple sclerosis cohort study. *EBioMedicine*, *72*. https://doi.org/10.1016/j.ebiom.2021.103590

Vågberg, M., Norgren, N., Dring, A., Lindqvist, T., Birgander, R., Zetterberg, H., & Svenningsson, A. (2015). Levels and age dependency of neurofilament light and glial fibrillary acidic protein in healthy individuals and their relation to the brain parenchymal fraction. *PloS one*, *10*(8), e0135886. https://doi.org/10.1371/journal.pone.0135886

Vaillant, A. A. J., & Qurie, A. (2022). Interleukin. In *StatPearls [Internet]*. StatPearls Publishing. Bookshelf ID: NBK499840

Van Epps, P., Oswald, D., Higgins, P., Hornick, T., Aung, H., Banks, R., Wilson, B., Burant, C., Gravenstein, S., & Canaday, D. (2016). Frailty has a stronger association with inflammation than age in older veterans. *Immunity & Ageing*, *13*, 1-9. https://doi.org/10.1186/s12979-016-0082-z

van Munster, C. E., & Uitdehaag, B. M. (2017). Outcome measures in clinical trials for multiple sclerosis. *CNS drugs*, *31*, 217-236. https://doi.org/10.1007/s40263-017-0412-5

Vani, P., & Chitra, V. (2022). The Role of the Proinflammatory and Anti-inflammatory Cytokines in Multiple Sclerosis. *Biomedical and Pharmacology Journal*, *15*(1), 137-146. https://dx.doi.org/10.13005/bpj/2349

Varadhan, R., Yao, W., Matteini, A., Beamer, B. A., Xue, Q.-l., Yang, H., Manwani, B., Reiner, A., Jenny, N., & Parekh, N. (2014). Simple biologically informed inflammatory index of two serum cytokines predicts 10 year all-cause mortality in older adults. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *69*(2), 165-173. https://doi.org/10.1093/gerona/glt023

Vargas, D. L., & Tyor, W. R. (2017). Update on disease-modifying therapies for multiple sclerosis. *Journal of Investigative Medicine*, *65*(5), 883-891. https://doi.org/10.1136/jim-2016-000339

Varhaug, K. N., Torkildsen, Ø., Myhr, K.-M., & Vedeler, C. A. (2019). Neurofilament light chain as a biomarker in multiple sclerosis. *Frontiers in Neurology*, *10*, 338. https://doi.org/10.3389/fneur.2019.00338

Vega, S. R., Strüder, H. K., Wahrmann, B. V., Schmidt, A., Bloch, W., & Hollmann, W. (2006). Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain research*, *1121*(1), 59-65. https://doi.org/10.1016/j.brainres.2006.08.105

Wallin, M. T., Culpepper, W. J., Coffman, P., Pulaski, S., Maloni, H., Mahan, C. M., Haselkorn, J. K., Kurtzke, J. F., & Group, V. A. M. S. C. o. E. E. (2012). The Gulf War era multiple sclerosis cohort: age and incidence rates by race, sex and service. *Brain*, *135*(6), 1778-1785. https://doi.org/10.1093/brain/aws099

Walton, C., King, R., Rechtman, L., Kaye, W., Leray, E., Marrie, R. A., Robertson, N., La Rocca, N., Uitdehaag, B., & van Der Mei, I. (2020). Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS. *Multiple Sclerosis Journal*, *26*(14), 1816-1821. https://doi.org/10.1177/1352458520970841

Wang, H., Wu, M., Zhan, C., Ma, E., Yang, M., Yang, X., & Li, Y. (2012). Neurofilament proteins in axonal regeneration and neurodegenerative diseases. *Neural regeneration research*, *7*(8), 620. https://doi.org/10.3969/j.issn.1673-5374.2012.08.010

Wang, K., Song, F., Fernandez-Escobar, A., Luo, G., Wang, J.-H., & Sun, Y. (2018). The properties of cytokines in multiple sclerosis: pros and cons. *The American journal of the medical sciences*, *356*(6), 552-560. https://doi.org/10.1016/j.amjms.2018.08.018

Warburton, D. E., Gledhill, N., Jamnik, V. K., Bredin, S. S., McKenzie, D. C., Stone, J., Charlesworth, S., & Shephard, R. J. (2011). Evidence-based risk assessment and recommendations for physical activity clearance: Consensus Document 2011. *Applied Physiology, Nutrition, and Metabolism*, *36*(S1), S266-S298. https://doi.org/10.1139/h11-062

Waubant, E., Lucas, R., Mowry, E., Graves, J., Olsson, T., Alfredsson, L., & Langer-Gould, A. (2019). Environmental and genetic risk factors for MS: an integrated review. *Annals of clinical and translational neurology*, *6*(9), 1905-1922. https://doi.org/10.1002/acn3.50862

Welk, G. (2002). *Physical activity assessments for health-related research*. Human Kinetics. ISBN: 9780736037488, 0736037489

Wens, I., Keytsman, C., Deckx, N., Cools, N., Dalgas, U., & Eijnde, B. (2016). Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. *European journal of neurology*, *23*(6), 1028-1035. https://doi.org/10.1111/ene.12976

Wesnes, K., Myhr, K.-M., Riise, T., Cortese, M., Pugliatti, M., Boström, I., Landtblom, A.-M., Wolfson, C., & Bjørnevik, K. (2018). Physical activity is associated with a decreased multiple sclerosis risk: The EnvIMS study. *Multiple Sclerosis Journal*, *24*(2), 150-157. https://doi.org/10.1177/1352458517694088

Westerlind, H., Ramanujam, R., Uvehag, D., Kuja-Halkola, R., Boman, M., Bottai, M., Lichtenstein, P., & Hillert, J. (2014). Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden. *Brain*, *137*(3), 770-778. https://doi.org/10.1093/brain/awt356

White, L. J., & Castellano, V. (2008). Exercise and brain health—implications for multiple sclerosis: part 1—neuronal growth factors. *Sports medicine*, *38*, 91-100. https://doi.org/10.2165/00007256-200838020-00001

White, L. J., Castellano, V., & Mc Coy, S. C. (2006). Cytokine responses to resistance training in people with multiple sclerosis. *Journal of sports sciences*, *24*(8), 911-914. https://doi.org/10.1080/02640410500357036

Wiendl, H., Gold, R., Berger, T., Derfuss, T., Linker, R., Mäurer, M., Aktas, O., Baum, K., Berghoff, M., & Bittner, S. (2021). Multiple Sclerosis Therapy Consensus Group (MSTCG): position statement on disease-modifying therapies for multiple sclerosis (white paper). *Therapeutic advances in neurological disorders*, *14*, 17562864211039648. https://doi.org/10.1177/17562864211039648. eCollection 2021

Williams, L., & Wilkins, L. (2009). ACSM's guidelines for exercise testing and prescription. *American College of Sports Medicine*, 152-180.

Wills, O. C., & Probst, Y. C. (2022). Understanding lifestyle self-management regimens that improve the life quality of people living with multiple sclerosis: a systematic review and meta-analysis. *Health and Quality of Life Outcomes*, *20*(1), 1-28. https://doi.org/10.1186/s12955-022-02046-1

Wingerchuk, D. M. (2012). Smoking: effects on multiple sclerosis susceptibility and disease progression. *Therapeutic advances in neurological disorders*, *5*(1), 13-22. https://doi.org/10.1177/1756285611425694

Wingerchuk, D. M., & Carter, J. L. (2014). Multiple sclerosis: current and emerging disease-modifying therapies and treatment strategies. Mayo Clinic Proceedings. https://doi.org/10.1016/j.mayocp.2013.11.002

Wu, J., Olsson, T., Hillert, J., Alfredsson, L., & Hedström, A. K. (2023). Influence of oral tobacco versus smoking on multiple sclerosis disease activity and progression. *Journal of Neurology, Neurosurgery & Psychiatry*. https://doi.org/10.1136/jnnp-2022-330848

Yalachkov, Y., Schäfer, J. H., Jakob, J., Friedauer, L., Steffen, F., Bittner, S., Foerch, C., & Schaller-Paule, M. A. (2022). Effect of estimated blood volume and body mass index on GFAP and NfL levels in the serum and CSF of patients with multiple sclerosis. *Neurology: Neuroimmunology & Neuroinflammation*, *10*(1), e200045. https://doi.org/10.1212/NXI.000000000020004

Yan, Y., Jensen, K., & Brown, A. (2007). The polypeptide composition of moving and stationary neurofilaments in cultured sympathetic neurons. *Cell motility and the cytoskeleton*, *64*(4), 299-309. https://doi.org/10.1002/cm.20184

Yang, J., Hamade, M., Wu, Q., Wang, Q., Axtell, R., Giri, S., & Mao-Draayer, Y. (2022). Current and future biomarkers in multiple sclerosis. *International Journal of Molecular Sciences*, *23*(11), 5877. https://doi.org/10.3390/ijms23115877

Yang, J. H., Rempe, T., Whitmire, N., Dunn-Pirio, A., & Graves, J. S. (2022). Therapeutic advances in multiple sclerosis. *Frontiers in Neurology*, *13*, 824926. https://doi.org/10.3389/fneur.2022.824926

You, Z., Luo, C., Zhang, W., Chen, Y., He, J., Zhao, Q., Zuo, R., & Wu, Y. (2011). Pro-and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. *Behavioural brain research*, *225*(1), 135-141. https://doi.org/10.1016/j.bbr.2011.07.006

Yu, Y., & Zheng, S. (2019). Research progress on immune aging and its mechanisms affecting geriatric diseases. *Aging Medicine*, *2*(4), 216-222. https://doi.org/10.1002/agm2.12089

Yuan, A., Rao, M. V., & Nixon, R. A. (2012). Neurofilaments at a glance. *Journal of cell science*, *125*(14), 3257-3263. https://doi.org/10.1242/jcs.104729

Zeydan, B., & Kantarci, O. H. (2020). Impact of age on multiple sclerosis disease activity and progression. *Current neurology and neuroscience reports*, *20*, 1-7. https://doi.org/10.1007/s11910-020-01046-2

Zhang, J.-M., & An, J. (2007). Cytokines, inflammation and pain. *International anesthesiology clinics*, *45*(2), 27. https://doi.org/10.1097/AIA.0b013e318034194e

Zhao, Y., Arceneaux, L., Culicchia, F., & Lukiw, W. J. (2021). Neurofilament light (NF-L) chain protein from a highly polymerized structural component of the neuronal cytoskeleton to a neurodegenerative disease biomarker in the periphery. *HSOA journal of alzheimer's & neurodegenerative diseases*, *7*(2). https://doi.org/10.24966/AND-9608/100056

Zielinski, M. R., & Gibbons, A. J. (2022). Neuroinflammation, sleep, and circadian rhythms. *Frontiers in Cellular and Infection Microbiology*, *12*, 853096. https://doi.org/10.3389/fcimb.2022.853096. eCollection 2022

Ziemssen, T., Akgün, K., & Brück, W. (2019). Molecular biomarkers in multiple sclerosis. *Journal of neuroinflammation*, *16*(1), 272. https://doi.org/10.1186/s12974-019-1674-2

Ziemssen, T., Bhan, V., Chataway, J., Chitnis, T., Cree, B. A. C., Havrdova, E. K., Kappos, L., Labauge, P., Miller, A., & Nakahara, J. (2023). Secondary progressive multiple sclerosis: A review of clinical characteristics, definition, prognostic tools, and disease-modifying therapies. *Neurology-Neuroimmunology Neuroinflammation*, *10*(1). https://doi.org/10.1212/NXI.0000000000200064

Zimmer, P., Bloch, W., Schenk, A., Oberste, M., Riedel, S., Kool, J., Langdon, D., Dalgas, U., Kesselring, J., & Bansi, J. (2018). High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial. *Multiple Sclerosis Journal*, *24*(12), 1635-1644. https://doi.org/10.1177/1352458517728342

Zivadinov, R., Weinstock-Guttman, B., Hashmi, K., Abdelrahman, N., Stosic, M., Dwyer, M., Hussein, S., Durfee, J., & Ramanathan, M. (2009). Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. *Neurology*, *73*(7), 504-510. https://doi.org/10.1212/WNL.0b013e3181b2a706

4 Appendices

4.1 Ethics Approval

HREB - Approval of Ethics Renewal 20161208
administrator@hrea.ca
Sent: Monday, March 06, 2023 2:35 PM
To: Stefanelli Mark(Principal Investigator) [cstefanelli@nl.rogers.com]
Cc: Ploughman Michelle(Co-Principal Investigator) [mploughm@mun.ca]; Moore, Craig; Hreaadministrator

Researcher Portal File #: 20161208

Dear Dr. Mark Stefanelli:

This e-mail serves as notification that your ethics renewal for study HREB # 2015.103 – Health Research Innovation Team in Multiple Sclerosis (HIT MS) Provincial Portfolio – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from 30 Apr 2023 to 30 Apr 2024.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an "Event".

The ethics renewal will be reported to the Health Research Ethics Board at their meeting dated 09 Mar 2023.

Thank you,

Research Ethics Office

(e) info@hrea.ca
(t) 709-777-6974
(f) 709-777-8776
(w) www.hrea.ca
Office Hours: 8:30 a.m. – 4:30 p.m. (NL TIME) Monday-Friday