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DIMETHYL FUMARATE FOR TREATMENT OF MULTIPLE SCLEROSIS: CLINICAL EFFECTS AND MECHANISMS

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Dimethyl Fumarate for Treatment of Multiple Sclerosis: Clinical Effects and Mechanisms
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To my parents and aunt Anita

ABSTRACT

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS). Dimethyl fumarate (DMF) is one of the more recent additions to a rapidly expanding treatment repertoire for MS. While DMF has proven beneficial for relapsing-remitting MS (RRMS) patients, its clinical profile in relation to current alternatives as well as its immunological effects are less known.

The overarching aim of the thesis was to assess the clinical effects of DMF for MS patients and investigate the underlying immunological mechanisms. Since both DMF and physical exercise is known to elicit an antioxidative response through the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), we further explored their immunological commonalities.

In Paper I, we showed that treatment discontinuations with DMF were lower than with interferons, the main existing initial drug choice, among newly diagnosed MS patients in Stockholm and Västerbotten Counties. Risks of having persistent disease activity, as shown by relapses and/or magnetic resonance imaging (MRI), were similar to fingolimod and natalizumab; two more recent disease modulatory therapies (DMTs). The main finding of the article, however, was that the comparator treatment, rituximab (Mabthera®; RTX), had a superior clinical effect compared to all other DMTs in terms of both clinical effect and treatment discontinuation.

In Paper II, we used Swedish nationwide data to compare DMF to interferons and glatiramer acetate, two common initial DMT choices, and fingolimod, which mainly is used as an escalation treatment. DMF proved more effective and had better drug survival in the first line comparison with interferons and glatiramer acetate but was less well tolerated than fingolimod when used second line.

In Paper III, we explored the immunological mechanisms of DMF treatment in humans underlying the clinical effects we observed in Paper I and II. We observed that DMF increased production of reactive oxygen species (ROS) in monocytes and that methylation changes occurred earlier in monocytes than in T cells. In addition, monocyte counts and levels of oxidized fat in blood were higher among treatment responders compared to non-responders, supporting the notion that DMF act by increasing oxidative burst in myeloid cells.

In Paper IV, we investigated the effects of aerobic exercise of moderate and high intensity on immune protein markers and kynurenine pathway (KP) metabolites in cerebrospinal fluid (CSF) and plasma of healthy participants. Participants in the high intensity group displayed changes in concentration of several immune markers and KP metabolites in both CSF and plasma, whereas participants in the moderate intensity group displayed few changes, suggesting a dose-response relationship. A separate comparison with DMF treated MS patients revealed few overlapping immune markers despite indications of overlapping mechanisms.

In conclusion, by affecting Nrf2 and oxidative burst, DMF has a unique mode of action among existing DMT options for RRMS, however, with limited overlap to effects mediated by physical exercise. Its clinical effectiveness is superior to traditional DMTs for newly diagnosed patients, but inferior to RTX. As an escalation DMT, it is less well tolerated than existing alternatives.

LIST OF SCIENTIFIC PAPERS

I. **Comparative effectiveness of rituximab and other initial treatment choices for multiple sclerosis.**
Mathias Granqvist, Malin Boremalm, Amyar Poorghobad, Anders Svenningsson, Jonatan Salzer, Thomas Frisell, Fredrik Piehl.
JAMA Neurology. 2018 Mar 1;75(3):320-327.

II. **Comparative effectiveness of dimethyl fumarate as the initial and secondary treatment for MS.**
Mathias Granqvist, Joachim Burman, Martin Gunnarsson, Jan Lycke, Petra Nilsson, Tomas Olsson, Peter Sundström, Anders Svenningsson, Magnus Vrethem, Thomas Frisell, Fredrik Piehl.
Multiple Sclerosis Journal. 2019 Aug 8:1352458519866600.

III. **Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis associates with ROS pathway in monocytes.**
Karl Carlström, Ewoud Ewing, **Mathias Granqvist***, Alexandra Gyllenberg*, Shahin Ainehband, Sara Lind Enoksson, Antonio Checa, Tejaswi Badam, Jesse Huang, David Gomez-Cabrero, Mika Gustafsson, Faiez Al Nimer, Craig Wheelock, Ingrid Kockum, Tomas Olsson, Maja Jagodic, Fredrik Piehl.
*These authors contributed equally to this work.
Nature Communications. 2019 Jul 12;10(1):3081.

IV. **Effects from physical exercise on measured soluble immune markers and kynurenine pathway metabolites in the intrathecal and peripheral compartment in healthy subjects.**
Josef Isung, **Mathias Granqvist**, Ada Trepci, Jesse Huang, Lilly Schwieler, Marie Kierkegaard, Sophie Erhardt, Jussi Jokinen, Fredrik Piehl.
Manuscript.

LIST OF SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

I. **The autoimmune spectrum of myasthenia gravis: a Swedish population-based study**
Fang Fang, Olafur Sveinsson, Gylfi Thormar, **Mathias Granqvist**, Johan Askling, Ingrid Lundberg, Weimin Ye, Lennart Hammarström, Ritva Pirskanen-Matell, Fredrik Piehl.
Journal of Internal Medicine. 2015 May;277(5):594-604.

II. **Successful combined targeting of B- and plasma cells in treatment refractory anti-NMDAR encephalitis.**
Olafur Sveinsson, **Mathias Granqvist**, Yngve Forslin, Kaj Blennow, Henrik Zetterberg, Fredrik Piehl.
Journal of Neuroimmunology. 2017 Nov;15(312):15-18.

III. **Depression and fatigue in multiple sclerosis: Relation to exposure to violence and cerebrospinal fluid immunomarkers**.
Philip Brenner, **Mathias Granqvist**, Johan Königsson, Faiez Al Nimer, Fredrik Piehl, Jussi Jokinen.
Psychoneuroendocrinology. 2018 Mar;89:53-58.

IV. **A novel, robust method for quantification of multiple kynurenine pathway metabolites in the cerebrospinal fluid.**
Lilly Schwieler, Ada Trepci, Stanislaw Krzyzanowski, Sigurd Hermansson, **Mathias Granqvist**, Fredrik Piehl, Tomas Venckunas, Marius Brazaitis, Sigitas Kamandulis, Daniel Lindqvist, Arthur Daniel Jones, Sophie Erhardt, Lena Brundin.
Bioanalysis. 2020 Mar;12(6):379-392.

CONTENTS

LIST OF ABBREVIATIONS

APC	Antigen presenting cell
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
CCL	Chemokine (C-C motif) ligand
CD	Cluster of differentiation
CIS	Clinically isolated syndrome
CSF	Cerebrospinal fluid
CXCL	Chemokine (C-X-C motif) ligand
DMF	Dimethyl fumarate
DMT	Disease modifying therapy
EAE	Experimental autoimmune encephalitis
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
FGF	Fibroblast growth factor
Gd^{+}	Gadolinium-enhancing lesion
GSH	Glutathione
HCAR2	Hydroxycarboxylic acid receptor 2
HIF-1α	Hypoxia-inducible factor 1-alpha
HLA	Human leukocyte antigen
IFN-γ	Interferon gamma
IL	Interleukin
JCV	John Cunningham virus
Keap1	Kelch-like ECH-associated protein 1
KYNA	Kynurenic acid
LAP	Latency associated peptide
MAGNIMS	Magnetic resonance imaging in multiple sclerosis
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MS	Multiple sclerosis

NF-ϰB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFL	Neurofilament light
NK	Natural killer
NOX	NADPH oxidase
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
OCB	Oligoclonal bands
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PML	Progressive multifocal leucoencephalopathy
PPMS	Primary progressive multiple sclerosis
RIS	Radiologically isolated syndrome
ROS	Reactive oxygen species
RRMS	Relapsing-remitting multiple sclerosis
RTX	Rituximab
SPMS	Secondary progressive multiple sclerosis
T_{cm}	T central memory cell
T_{em}	T effector memory cell
TGF	Transforming growth factor
T_h	Helper T cell
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

1 MULTIPLE SCLEROSIS

MS is a chronic autoimmune and degenerative disease of the CNS with recurring bouts of inflammatory activity, over time often resulting in accumulating neurological deficit that impedes activities of daily life (1). It is the most common cause of non-traumatic neurological deficits in young adults in Sweden, with an annual incidence of 10 per 100.000 and a prevalence of 189 per 100.000 with a female to male ratio of 2.3:1 (2). An estimated 2.5 million people are suffering from MS worldwide, with an average increase in incidence of 10.4% between 1990 and 2016 (3). The majority (85%) of incident cases of MS are in the form of RRMS, which is characterized by acute onsets of neurological deterioration, often debuting between ages 20 and 40, interleaved by varying degrees of recovery (1). Earlier findings have shown that about half of patients convert to secondary progressive MS (SPMS) within 15 to 20 years (4). SPMS is hallmarked by a steady disease progression and is usually treatment refractive in proportion to absence of signs of inflammatory disease activity as shown by overlaid relapses or accumulation of lesions on MRI. Some patients debut with this symptomatology, in which case it is referred to as primary progressive MS (PPMS) and constitutes about 5% of MS patients. PPMS usually debuts in middle-aged persons and is proportionally equally distributed between the two sexes. Life expectancy of MS patients is generally 7-14 years shorter (5,6), although this has been shown to be at least partially mitigated by use of DMTs (7,8).

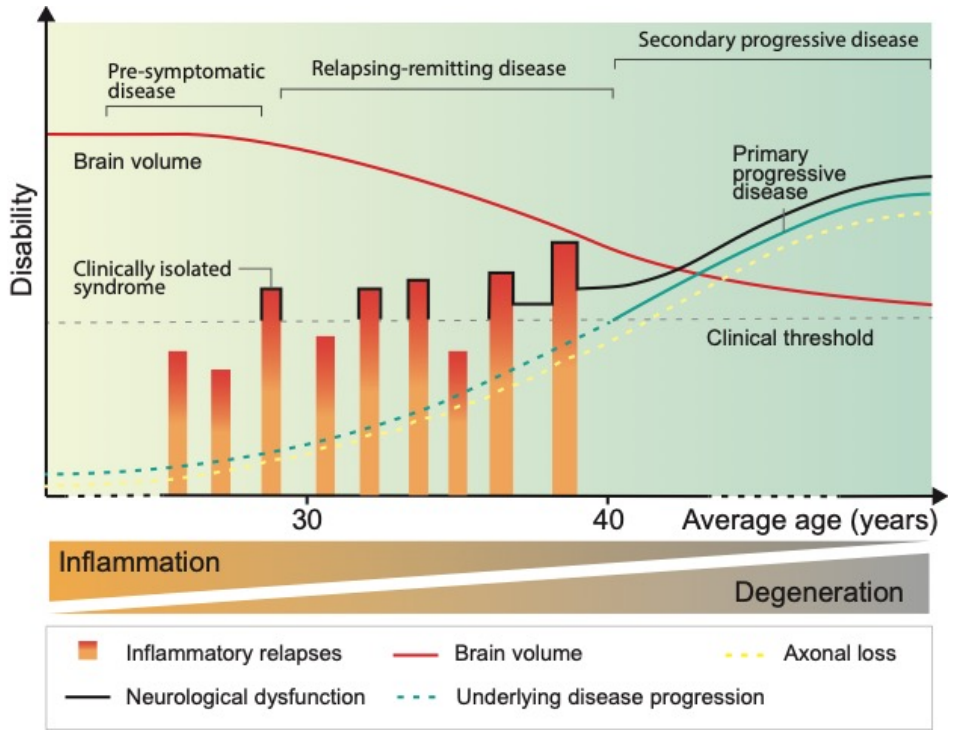

Figure 1. Typical disease course of patients with multiple sclerosis (MS). Clinical symptoms of MS usually present in the third and fourth decade, displaying wide heterogeneity although the disease is speculated to begin several years prior. The disease usually initially manifests as relapsing-remitting (RRMS) which later turns into a progressive accumulation of deficits. While inflammation dominates the early disease course, degenerative mechanisms dominate the latter.

2 ENVIRONMENTAL AND GENETIC RISK FACTORS FOR MS

Comparing incidence rates across countries has led to the observation of a general latitudinal gradient of increasing disease occurrence both north and south of the equator (9), although with debated exceptions within Western Europe, North America and parts of the Mediterranean (10,11). There is also generally a higher incidence in Western countries compared to Asia, Oceania and Africa (10). Potential underlying factors to this gradient include UV-exposure, vitamin D and melatonin levels. Low levels of UV-exposure, dietary vitamin D and vitamin D in serum have been associated with an increased risk of developing MS (12,13). Despite serum vitamin D levels correlating with disease activity in MS, supplementation has not shown any therapeutic effect when disease is manifest or in preventing conversion to clinically definitive MS (14–16). Furthermore, increased relapse rates have been observed during the darker winter months (17), which correlates to generally increased levels of melatonin, the secretion of which is in part inhibited by retinal exposure to blue light. Melatonin levels in have been shown to be negatively correlated with MS relapses in humans and mediate the severity of experimental autoimmune encephalomyelitis (EAE), an animal model of MS (18). Migration studies have also shed light on the importance of geographical location at different developmental stages. Migrating from a country with low incidence to one with high incidence during adolescence increases the risk of MS to levels comparable with that of the residential population (19). Further environmental risk factors for MS include Epstein-Barr virus (EBV) infection, exposure to tobacco smoke and organic solvents, night-shift work and obesity in adolescence whereas seropositivity for CMV infection, use of oral tobacco, alcohol consumption and high coffee consumption have been associated with a lower risk of disease development (20–24). Diet has long been speculated to be a contributing factor in MS development, however, aside from a possible benefit of vitamin D supplementation, no conclusive contributory role has been found (25). The risk of developing MS attributed to genetic factors has been reduced compared to earlier estimates, with a 17% concordance rate between monozygotic twins according to recent studies (26). Genome wide association studies have identified over 200 risk loci underlying the predisposition for developing MS, most of which are associated with regulation of the immune system (27,28). The most well-characterized associated allele is the human leukocyte antigen (HLA) class II DRB1*15:01, encoding the major histocompatibility complex II (MHC II), which confers a three times increased risk for development of MS. The HLA class I allele A*2:01, on the other hand, is protective against MS. When combining known genetic and environmental risk factors, the risk of developing MS increases notably, indicating a significant gene-environment interaction (23).

3 MS PATHOLOGY

The immune system is developmentally conditioned to protect against disease. Immune system recognition and interaction with host cells, autoimmunity, is an important part of this function. Autoimmune disease defined as the result of aberrant immune interactions, which has been shown in several aspects of MS pathology.

3.1 ADAPTIVE IMMUNE SYSTEM IN MS

The adaptive immune system is responsible for elimination of pathogens with high specificity through several adaptable mechanisms. What distinguishes the cell lineages of the adaptive immune system is the development of highly specific receptors that can target a wide range of antigens through a process called V(D)J recombination which allows a great number of possible receptor genes through rearrangement of sets of receptor gene segments. The two major cell types of the adaptive immune system are T cells and B cells, each with distinct functions. Both of these lymphocyte subsets can be further divided based on functionality and surface markers such as cluster of differentiation (CD). $CD4^+$ T cells and $CD8^+$ T cells have been central to the current understanding of MS pathology and will be further elaborated upon below.

3.1.1 T cells

$CD4^+$ T cells, also known as T helper cells (T_h cells), are instrumental in orchestrating a range of immune responses. Derived from hematopoietic precursors in the bone marrow, T cells develop and mature through a series of positive and negative selection based on their T cell receptor affinity and specificity in the thymus. $CD4^+$ T cells are activated by receptor binding to peptides presented on MHC class II by antigen presenting cells (APCs) and can then differentiate into different $CD4^+$ T cell subsets including T_h1, T_h2, T_h17 and T regulatory (T_{reg}) cells depending on composition of co-stimulatory molecules. When activated, $CD4^+$ T cells support effector cells such as macrophages and $CD8^+$ T cells, which in turn are responsible for phagocytosis and clearing of cellular debris as well as cell mediated cytotoxicity. Adoptive transfer of myelin specific $CD4^+$ T cells in rodents is sufficient to result in EAE. Supported by this causal role in EAE, $CD4^+$ T cells have long been seen as a key component in MS pathogenesis. T_{reg} cells, a subset of $CD4^+$ T cells expressing CD4, forkhead box P3 and CD25 are driven by transforming growth factor (TGF)-β signaling, are considered important in suppressing inflammation and self-reactivity in MS (29).

$CD8^+$ T cells are important for defense against intracellular infections and elimination of damaged cells. Their primary effector functions are release of cytotoxins such as granzymes, and perforins that induce cell death. They also secrete the proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). Intrathecal levels of $CD8^+$ T cells are correlated with myelin destruction axonal degeneration in MS lesions (30). Subsets of $CD8^+$ T cells include central memory and effector memory T cells (T_{cm} and T_{em}, respectively), which retain cytotoxic capabilities and are thought to provide protection against chronic infections (T_{cm}) and certain viruses and bacteria (T_{em}) (31).

3.1.2 B cells

Since the clinical efficacy of B cell-depleting therapies such as RTX and ocrelizumab has become evident (see Paper I), interest in the immunopathological role of B cells in MS has risen sharply and altered its emphasis. The antibody production of plasma B cells has historically been of much interest due to their increased production of a narrow set of immunoglobulins (Ig), termed oligoclonal bands (OCB), and are highly correlated with elevated IgG levels intrathecally (32). Although OCBs occurs in other diseases, such as neuromyelitis optica, neurosarcoidosis, syphilis and multiple myeloma, as mentioned earlier, it is included as a criterium for an MS diagnosis. Defining common CNS targets of these Igs between patients has with current efforts been elusive as they are usually specific to ubiquitously occurring autoantigens. Further, immunoglobulin reactivity to myelin oligodendrocyte glycoprotein and other plausible CNS specific molecules does not differ significantly between MS patients and controls (33). Focus has thus shifted to the pathogenic influence of the B cells' cytokine release and role as an APC (34). B cells has recently been found to interact with T cells in a HLA-DR-dependent manner to support autoproliferation of T cells likely to target an autoantigen present in brain parenchyma (35). Clinically, the MS biomarker chemokine (C-X-X motif) ligand(CXCL)13 functions as a chemoattractant for B cells and CSF levels correlate both with markers of disease activity and can be used to predict treatment response (36,37).

3.2 INNATE IMMUNE SYSTEM IN MS

The innate immune system constitutes the first line of defense against various pathogens, carrying fixed and unadaptable repertoires of receptors named pattern recognition receptors. Recognition of antigens by these receptors lead to a release of several proinflammatory proteins such as cytokines interleukin (IL)-1, TNF-α and IFN-γ, collectively inducing the classic signs of inflammation. The innate and adaptive immune system cooperates through cytokine signaling, antigen presentation on MHC class II molecules and, possibly, production of ROS. Of the many components of the innate immune system, monocytes and macrophages are the most relevant within the confines of this thesis.

3.2.1 Monocytes and macrophages

The main effector functions of monocytes and macrophages are antigen presentation, phagocytosis and cytokine production. Common cytokines produced by monocytes include IL-1, IL-12 and TNF-α. In turn, IFN-γ, often secreted by $CD4^+$ T cells, are necessary for macrophage activation and phagocytosis, whereas chemokine (C-C motif; CCL)2 and CCL7 are typical factors regulating monocyte homing. The surface markers CD14 and CD16 are often used to distinguish subpopulations of monocytes, from classical ($CD14^+CD16^-$) to non-classical ($CD14^+CD16^{++}$) and intermediate ($CD14^{++}CD16^+$) monocytes. Monocyte ROS production is hypothesized to be a regulatory factor in the interplay with $CD4^+$ T cells, as impaired ROS production has been associated with chronic inflammation (38).

3.3 PATHOLOGICAL CHARACTERISTICS OF MS

Characteristic pathological features of MS encompass demyelination in both white and grey matter, axonal degeneration, reactive gliosis and increased vascular permeability at lesion sites in the described typical locations. In active MS lesions, there is perivascular and parenchymal infiltration of macrophages (histologically described as a sea of macrophages), T cells ($CD8^+$ and $CD4^+$), B cells and plasma cells in decreasing order. Resident astrocytes swell up and form multinucleated hypertrophic gemistocytes (39) creating a matrix in which the other cells are suspended. Oligodendrocytes, responsible for the neuronal myelin sheaths are usually damaged in various degrees, but also show signs of active remyelination (40). Ig depositions and complement activation is often present, which in conjunction with the previously described give rise to a heterogenic pattern of lesions that can be categorized into distinct categories which vary between patients (41). ROS production by infiltrating macrophages and resident microglia contribute to myelin damage, though much point towards ROS also being necessary for regulation of inflammation through $CD4^+$ T cell signaling (38,42). Several hypotheses have been proposed regarding the precipitating event in MS and can roughly be divided based on whether it is thought to be initiated peripherally or in the central nervous system (CNS). Molecular mimicry, epitope spreading and bystander activation are some of the hypotheses that have been proposed as an "outside-in" mechanism (43–45). Activated by the recognition of a self-antigen, $CD4^+$ T cells are thought to differentiate peripherally to T_h1 and T_h17 cells, cross the blood-brain barrier (BBB) and propagate an autoimmune reaction through recruitment of monocytes and further activation of $CD4^+$ T cells and local microglia (46). In the "inside-out" model, the autoimmune response is considered secondary to a degenerative process within the CNS. Neuroaxonal degeneration has been shown to occur already in early stages of MS and is not dependent on neuronal demyelination (47).

In SPMS, meningeal ectopic germinal centers, containing follicular dendritic cells, B cells and plasma cells, have been described, which might secrete soluble factors driving cortical demyelination (46). Wide-spread activation of resident microglia has also been observed, capable of further driving the disease process through secretion of ROS, complement factors and inflammatory cytokines (48).

4 CLINICAL ASPECTS OF MS

4.1 MS SYMPTOMS

MS patients experience symptoms across many different functional systems depending on the locations new lesions appear. Initial symptoms often include vision disturbances, sensory abnormalities such as numbness or paresthesia and muscle spasms or weakness which is complicated by difficulties with balance and coordination and musculoskeletal pain leading to impairments in mobility. With time, many patients often also experience cognitive and speech deficits, mood disorders and bladder and bowel problems. Fatigue is one of the most common symptoms in MS, occurring in up to 90% of patients (49). While there's no set definition of fatigue, most patients describe it as a "reversible motor and cognitive impairment, with reduced motivation and desire to rest" (50), and about 40% of patients find it is their most debilitating symptom. Fatigue correlates with depression, low quality of life, reduced activities of daily life and physical activity with a likely bidirectional influence (51,52).

4.2 MS DIAGNOSIS

Clinically, RRMS is diagnosed by assessing relapses, which are acute focal neurological deficits associated with immune-mediated demyelination lasting at least 24 hours and with varying rates of functional recovery (53). MRI has not only had a profound effect on the understanding of the development of MS pathology over time, it has also become a main pillar in the diagnosis of MS, showing evidence of ongoing and past demyelination events as well as regional and general atrophy. It should be noted, however, that MS can be diagnosed without the use of an MRI. The diagnosis of MS is based on evidence of disease activity being dispersed in time and space (54). This means a patient must display symptoms of at least two lesions in the CNS occurring at separate occasions. Typical regions of the CNS that are affected are divided into cortical or juxtacortical, periventricular, infratentorial and spinal cord. The MRI diagnostic criteria from the recent MAGNIMS guidelines support optic neuritis counting as a separate lesion and allows presence of OCB in CSF to substitute for time dispersion of lesions in patients with clinically isolated syndrome (CIS) (55). Patients are diagnosed with CIS based on clinical evidence of a single relapse suggestive of MS. Presence of MRI lesions in these patients increase the risk of being diagnosed with MS from 20 to 90% (56). Treatment of CIS patients with DMTs also lowers the risk of further demyelinating events and conversion to definite MS (57,58). Patients incidentally presenting with MRI lesions suggestive of MS and no neurological manifestations are diagnosed with Radiologically isolated syndrome (RIS), of which 34% convert to MS over a 5-year period (59,60). The diagnosis of PPMS relies mainly on clinical parameters of disease accumulation, though also supported by the occurrence of OCB in the CSF and MRI lesions. PPMS and SPMS is often further divided into active and/or progressive depending on occurrence of relapses, MRI activity and progressive accumulation of functional deficits (61). Differential diagnoses that should be ruled out before MS is diagnosed include neuromyelitis optica, neurosarcoidosis, Lyme disease, acute demyelinating encephalomyelitis, cerebral vasculitis, Arnold-Chiari malformation, systemic lupus erythematosus and Sjögren's disease (62).

4.3 MS BIOMARKERS

Complementary to the clinical assessment and MRI, there are several biomarkers that reflect the disease processes of MS that are currently being evaluated for clinical usefulness. The biomarker garnering most attention in later years has been neurofilament light (NFL). NFL is an integral part of the axonal structure and is exuded into CSF and blood upon neuronal injury, which include inflammatory, degenerative and traumatic causes. In MS, CSF and serum NFL increases with both clinical relapses and Gd^+ MRI lesions, and correlates moderately with the clinical severity scale Kurtzke Expanded Disease Severity Score (EDSS), explained in greater detail in the next section (63–65). As a prognostic marker, high NFL levels correlates increased accumulation of disability and earlier conversion to SPMS (66). Other biomarkers include matrix metalloprotein (MMP)-9 in CSF and serum which correlated with disease activity and also distinguishes MS from other inflammatory neurological diseases (67). Osteopontin, a phosphoprotein found in extracellular matrix that interacts with several types of integrin receptors, correlate with MS disease and increase with disease activity (68). OCB, IgG-index and CXCL13 are biomarkers further discussed in sections 3.13 and 5.2.

4.4 MS FUNCTIONING SCALES

Several scales for measuring the clinical severity of MS have been proposed and are used to varying degrees between countries and clinics. The most widely recognized scale internationally is EDSS (69) which assesses impairment in eight functional systems including visual, brainstem, pyramidal (motor), cerebellar (coordination), sensory, bladder/bowel and cerebral (cognition) components. The scale uses an ordinal rating system in 0.5 increments (after EDSS 1) that ranges between 0 and 10, representing normal neurological status and death due to MS, respectively. Scores between 0 and 3.5 measures impairment in the defined functional systems, 4.0 to 7.0 focusing on walking ability and walking aids, and 7.5 to 9.5 dependence on help. The scale is most often used to measure disease outcomes in clinical trials, usually secondary to relapses and MRI lesions. EDSS correlates moderately to MRI lesion load, though is heavily weighted towards corticospinal lesions as they usually result in greater functional impairments (70). Correlations are, however, strengthened when combined with measures of brain atrophy (71).

4.5 MS COMORBIDITY

Comorbidities are common among patients with MS and vary over time. Among newly diagnosed patients, chronic lung disease (4.9%), hypertension (3.8%), hypercholesterolemia (2.7%), depression (7.4%) and anxiety (2.7%) occur most frequently (72). Rates of past and current obesity are also higher among MS patients at diagnosis (73). Across all MS patients, depression (23.7%), anxiety (21.9%), hypertension (18.6%) and hyperlipidemia (10.9%) are among the most commonly occurring comorbidities, based on a systematic review including studies from Europe (52.2%) and North America (33.7%). Over time, the prevalence of hypertension, diabetes, hyperlipidemia, ischemic heart disease and fibromyalgia tend to increase

(74). Occurrence of comorbidities have been associated with increased relapse rate, disease progression as well as earlier treatment discontinuation, often due to tolerance issues (74).

5 MS TREATMENT

The range of RRMS treatments has expanded rapidly and considerably since the introduction of interferons in 1993 (75). As of 2019, there were in total 18 approved DMTs for RRMS, which had undergone clinical testing using placebo or the oldest available DMTs as comparators in mostly treatment naïve patients, underscoring the need for comparative prospective trials between reasonable treatment alternatives (76). As a result, treatment strategies have in the past years varied significantly both between and within countries. In many countries, including the US and those comprising Europe, the most common strategy remains an escalation type strategy, starting with the oldest, least effective drugs (first line) and escalating to more efficient (second line) therapies when patients display signs of disease breakthrough. One of the main rationales for this being the lower risk of serious adverse events with the first line drugs such as interferons or glatiramer acetate (77,78). Replacing this strategy with using highly efficient therapies early on has been a topic of much discussion (79,80) and has in the past years been the prevailing strategy in most Swedish counties (see Paper I and II). Epidemiological findings seem to favor this strategy, showing relapse rate in the first two years influencing disease course and an increased time to disability milestones among patients with highly efficient therapies early in disease (81,82). A recent retrospective observational study which included patients from both the international MS registry, MSBase, and the Swedish MS registry, showed that patients treated with a highly efficient therapy within two years (in contrast to after four to six years) of clinical disease onset had significantly lower levels of disability at six and ten years follow-up (83). An induction type strategy, combining initial use of rituximab, a highly effective treatment, followed by subsequent continuous use of glatiramer acetate, a common first line therapy, showed lowered risk disease activity up to 30 months after treatment initiation compared to glatiramer acetate monotherapy (84). Included among highly efficient therapies are monoclonal antibodies rituximab, ocreluzumab (both targeting CD20), natalizumab ($\alpha 4\beta 1$ integrin) and alemtuzumab (CD52) along with mitoxantrone (a topoisomerase inhibitor). Not usually included when discussing highly effective treatments, but with corresponding or higher efficacy is autologous haemopoietic stem-cell transplantation, which in a phase II study showed up to 78-83% total absence of disease activity (85). Due to a comparatively high mortality rate (~5%), however, it usually reserved for patients with early aggressive disease activity refractory to highly effective treatment alternatives.

5.1 DIMETHYL FUMARATE

Initially developed as a treatment for psoriasis, DMF was by chance found to be effective also for RRMS patients and was approved for that indication in 2013 (86). Through the DEFINE and CONFIRM trials and their extension trial ENDORSE, DMF displayed a moderate efficiency for decreasing relapse rates and gadolineum contrast enhancing (Gd^+) and newly appearing T2 lesions and has since become the most prescribed RRMS drug in the US (87–

89). Early studies in EAE showed that DMF binds irreversibly to Kelsh-like ECH-associated protein 1 (Keap1), thereby increasing the activity of Nrf2, a central transcription factor for several antioxidative and cytoprotective enzymes, such as NADP(H) quinoline oxidoreductase-1 (NQO1) (90). Notably, Nrf2 activation is well described as a consequence of physical exercise (91). Despite these findings, evidence of CNS penetration in humans has not been shown, leading to speculation that it primarily exerts its effects through peripheral immunomodulation (92). DMF exerts a direct modulatory effect on microglia through activation of the hydroxycarbolic acid receptor 2 (HCAR2) resulting in inhibition of NF-κB and lower secretion of pro-inflammatory cytokines (93,94). As shown in Paper III, DMF treatment dependent increases in circulating monocytes and monocytic ROS production predict treatment response among patients (95). Insufficient treatment response was also correlated with the minor *G* allele of the NADPH oxidase (NOX)3 gene involved in ROS generation. DMF also affect circulating concentrations of leucocytes, reducing $CD8^+$ and $CD4^+$ T cells and subsets of central memory ($CD45RA^-$ $CCR7^+$) and effector memory ($CD45RA^-$ $CCR7^-$) T cells, Tc17 cells (IL-17 secreting $CD8^+$ T cells), $CD56^{dim}$ natural killer (NK) cells, $CD19^+$ B cells and plasmacytoid dendritic cells, while increasing naïve ($CD45RA^+$ $CCR7^+$) T cells and leaving T_{reg} cells unchanged (figure 2; 96–98). How this is regulated is unclear, though studies have shown DMF to induce apoptosis in active subsets of T cells (99). Through inhibition of glyceraldehyde 3-phosphate dehydrogenase (GADPH) and in extension aerobic glycolysis in activated immune cells, DMF promotes development of T_{reg} cells while inhibiting differentiation and proliferation of T_h1 and T_h17 cells (100). Human studies show low levels of DMF in circulating plasma after intake, whereas intracellular GSH levels rapidly decrease (101). Rapid depletion of intracellular glutathione (GSH) levels is hypothesized to generate an anti-inflammatory cytokine response or cell apoptosis. Decreased levels of GSH in T cells have been correlated with downregulation of T_h1 cytokines (102) supported by $CD4^+$ T cell production of IFN-γ and IL-17 having been found to decrease with DMF treatment whereas IL-4 production increases (97). DMF exerts a wider range of effects than those studied within this thesis, as reviewed by Yadav *et al*. (103). Clinically, adverse events such as flushing (believed to be HCAR2-mediated) and gastrointestinal upset are among the most common causes of discontinued use (see Paper I & II) (104). There have been case reports of patients suffering progressive multifocal leukoencephalopathy (PML) in association with DMF treatment, all cases with associated lymphocytopenia (105). Also seen among patients treated with natalizumab, PML is a disease caused by an opportunistic viral infection of the John Cunningham virus (JCV) which is present in approximately 40 to 60% of the population (106). One case has been reported with *Listeria Monocytogenes*-induced rhombencephalitis, a foodborne usually severe intracellular infection, with the patient having a slight reduction in lymphocyte count (107).

5.2 RITUXIMAB

Initially developed as a therapy for patients with non-Hodgkin's lymphoma, RTX has since gained an important role in the treatment of several diseases within hematology, rheumatology and neurology. The list of diseases under investigation for a therapeutic effect by RTX is still

increasing. Within clinical neurology, it has become widely used for neuroinflammatory diseases with off-label use for diagnoses including neuromyelitis optica, myasthenia gravis, N-methyl-D-aspartate receptor encephalitis and other autoimmune encephalitides (108–110). Pharmacodynamically, RTX is a chimeric monoclonal antibody against CD20 which is primarily expressed on B cells. In Paper I, we showed that RTX is a highly effective and safe treatment for RRMS. Other, further humanized CD20 antibody treatments have also been approved for clinical use and include ocrelizumab (90-95% humanized) and ofatumumab (fully humanized), the former with partially overlapping epitope and clinical efficacy in RRMS patients comparable to that of rituximab as shown in the OPERA I and II trials (111). RTX treatment leads to the lysis of $CD20^+$ B cells through antibody-dependent cellular cytotoxicity (mediated by carriers of Fcγ receptors such as monocytes, neutrophils and NK cells), complement-dependent cytotoxicity and apoptosis (112). CD20 is present on later stage developing B cells to mature and memory B cells, the latter a reservoir for EBV, a known risk factor for MS (113). Antibody producing plasma B cells, however, do not express CD20, and CSF OCBs remain despite therapeutic effect, leading to an exploration of antibody independent B cell contributions to MS disease (see section 3.1.2). CD20 has also been found in subpopulations of T cells, sparking debate on the main mode of action for anti-CD20 antibodies in autoimmune inflammatory diseases (114). The repopulating naïve B cells following RTX treatment secrete less proinflammatory cytokines (IL-6, TNF-α and granulocyte-macrophage colony-stimulating factor), and higher levels of IL-10 that mediates an anti-inflammatory effect on T and myeloid cells (115). RTX treatment has been associated with an increased susceptibility to serious infections compared to both moderate and highly effective treatment alternatives (116). Although cases of PML have been reported among RTX treated patients with other inflammatory and non-inflammatory diseases, occurrence is rare (117,118).

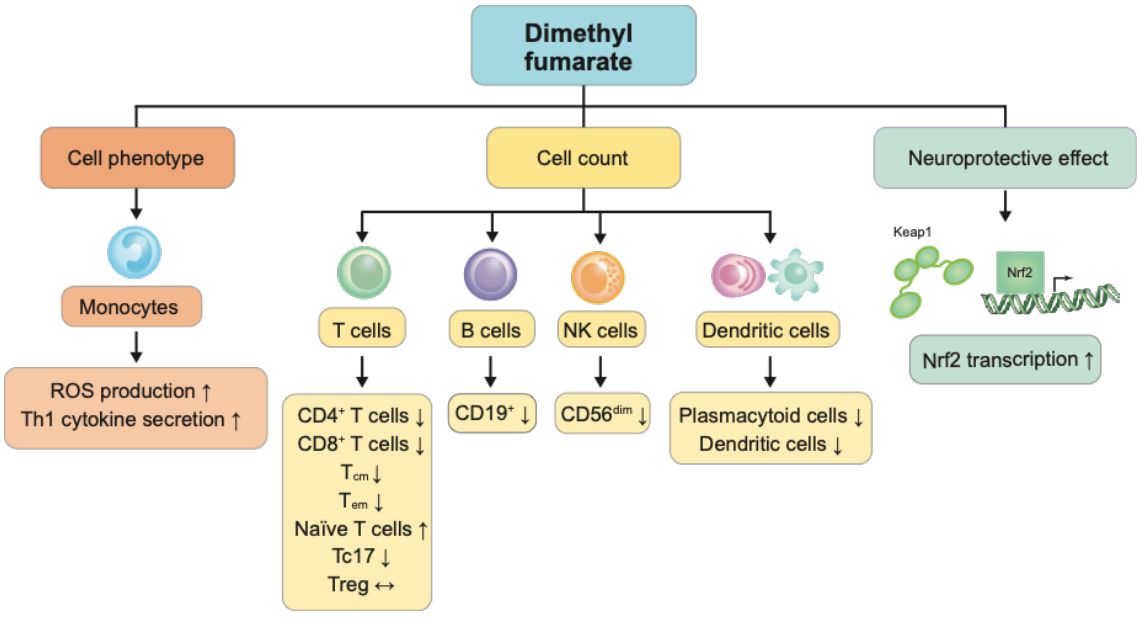

Figure 2. Schematic summary of the reviewed mechanisms for DMF. Dimethyl fumarate exerts a wide range of effects on immune cells and neurons. Changes in levels of T cell subsets such as T effector memory (T_{em}) and T central memory (T_{cm}) cells have been extensively described, although several other cell types are affected.

6 EXERCISE AND MS

The clinical approach to physical exercise for patients with MS has changed profoundly during the past century. Early on, absence of even moderately demanding physical activity was deemed critical for MS patients to avoid further neurological deterioration (119). This recommendation was influenced by the tendency of MS symptoms temporarily worsening with increased body temperature, typically brought on by heat exposure or physical exertion, known by its clinical term as Uhthoff's phenomenon. In 1963, however, a report by Geisler *et al*. (120) provided proof of successful outcomes from exercise related rehabilitation and research interest has been steadily increasing since. Accumulating evidence shows benefits of physical exercise along several functional domains for MS patients (reviewed in (121)). Despite this, MS patients are less likely to exercise (122) and participate in social/lifestyle activities (123), likely in part due to the high prevalence of fatigue along with motor and coordination deficits (51). Compared to healthy controls, MS patients across all subgroups are in poorer physical condition regarding aerobic capacity, maximal muscle strength, gait velocity and health-related quality of life (124). Areas of less well-established disease impact include increased resting heart rate, increased diastolic blood pressure, increased muscle atrophy, decreased muscle mass, decreased fat-free mass, and shifts in muscle fiber type (124). While DMTs reduce disease activity and disease progression, exercise remains the only way to improve function in MS, at least in the long term (82,125). Exercise can be classified into different types depending on the type of muscle contraction, intensity and duration. For the purposes of distinguishing exercise protocols for MS patients, the most commonly used are aerobic (endurance), strength, balance and stretching exercises. Exercise is also commonly grouped with other activities, such as rehabilitative exercises (strength and endurance exercise in combination with stretching, psychotherapy etc.) and occupational therapy (work-oriented activities and exercises) and is generally regarded as safe for MS patients (126). Recommendations regarding type, frequency and duration of exercise has been formulated in the Canadian Physical Activity Guidelines for Adults With Multiple Sclerosis (127). The guidelines are based on a systematic review of exercise literature and a consensus meeting with stakeholder involvement. As the impact of exercise on clinical parameters is becoming better understood, one of the current main challenges of the field is to optimize exercise protocols for specific functional deficits, as well as to understand the molecular basis of how the beneficial effects are mediated.

6.1 EXERCISE EFFECTS ON MS SYMPTOMS AND COMORBIDITY

Increasing evidence support the beneficial effects of exercise on ameliorating MS symptoms, with varying efficiency depending on exercise type. Benefited areas of function involve walking mobility, balance, cognition, fatigue, depressive symptoms and health related quality of life (128–131). Also, comorbidities commonly associated with MS (described prior) benefit significantly from exercise (132).

6.2 EXERCISE EFFECTS ON MS IMMUNOLOGY

Aside from the ameliorating effects on many symptoms, accumulating evidence shows that exercise also likely has a modulating effect on inflammation in MS (133,134). Epidemiological studies partially support the notion of a protective effect of exercise on MS incidence (135,136) and disease burden (137). A systematic review of relapses in exercise studies found a 27% decrease of relapses in exercise intervention groups compared to controls, whereas retrospective epidemiological studies have not shown a correlation (138,139). Premorbid activity levels and peak aerobic capacity predict disease progression in MS, though resistance exercise did not ameliorate rate of brain atrophy or decrease lesion load despite increasing cortical thickness and functional scores (140–142). Although long-term effects of exercise in MS patients have not been studied, reductions in disability accumulation and neuronal loss are assumed based on current experimental findings, represented schematically in figure 3 (143). However, exercise has not been found to influence peripheral levels of T cells, B cells, NK cells or monocytes in patients with IFN-b treatment (144).

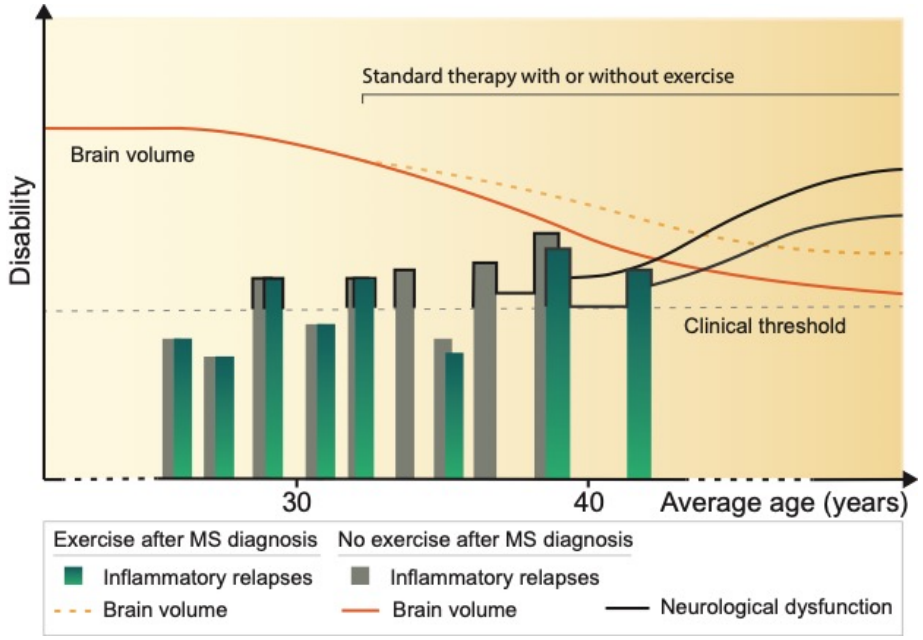

Figure 3. Hypothesized long-term clinical effects of exercise added to standard MS therapy. The normal course of disease progression is marked in grey bars, with a corresponding exercised disease course overlaid in green bars. Observed benefits of exercising MS patients include lower relapse rate (as shown by the green bars) and, speculatively based on EAE findings, slower accumulation of disabilities.

6.2.1 Immunomodulatory effects of exercise in EAE

Much of the current understanding of the immunological aspects of MS has been derived from animal models, predominantly from rodents. Of the experimental models of MS, EAE is the most commonly used. Though there are significant discrepancies between the disease course and lesion distribution and disease driving cell subsets, the pattern of demyelination and axonal damage is highly similar (145,146). Studies on EAE have shown that intense, but not moderate,

exercise can delay the onset of disease, time to maximum disease severity and decrease duration of disease exacerbation (147–149). It did not, however, have an influence on maximal disease severity (147,150). Timing has been shown crucial for the immunomodulatory effects of exercise. Exercise prior to disease induction had no influence on the disease course, whereas exercise after induction delayed disease onset (150). Since exercise interventions are initiated after diagnosis, i.e. after the patient has incurred a neurological deficit, Motl *et al*. initiated their exercise intervention at the remission of the first relapse. In this setting, exercise had no influence on disability scores or hippocampal brain-derived neurotrophic factor (BDNF) (151). Adoptive transfer of T cells from intensely exercised EAE mice resulted in a milder disease course in recipients. The same T cells proliferate in lower rates and had lower production of TNF-α, IFN-γ, IL-17 and IL-10 after exposure to myelin components *in vitro* (148). Lower levels of IFN-γ, IL-17 and IL-1β in spinal cord was also found after exercise (149).

6.2.2 Exercise and cytokines in MS

Cytokines are short peptides with various cell-signaling functions, importantly including immune modulation. Several cytokines have been of interest in MS due to findings either from clinical samples or studies of EAE. TNF-α was thought to be an important promoter of MS given that levels were increased in the CSF of patients and were correlated with disease progression and blockade in EAE resulted in significant reduction in disease severity (152,153). Unfortunately, clinical trials saw an increase in disease activity among patients receiving anti TNF-α antibody treatment (154). CXCL13 is important for B cell homing to CNS and levels are predictive of disease exacerbations and prognosis (155). Several other cytokines have been detected among MS patients, including IL-6, CCL4, CCL22, CXCL10 (156,157), but inconsistencies across studies remain problematic. This also pertains to cytokines associated with exercise interventions in MS (133). Cytokines TNF-α, IL-6 and IL-10, which have been implicated in MS all show inconsistent changes in peripheral concentrations (158–161).

6.2.3 Exercise and neurotrophic factors in MS

Neurotrophic factors are important mediators for cell survival and synaptic plasticity and have been associated with both exercise and MS pathology (162). Increased levels of nerve growth factor receptors have been found in active MS lesions and on microglia/macrophages (163). Brain-derived neurotrophic factor (BDNF) is increased in the relapse and recovery phase of RRMS and is decreased in SPMS. Basal levels of serum nerve growth factor are increased in MS patients and are further increased after acute aerobic exercise compared to healthy controls. BDNF concentrations increased with exercise but not more so than for controls in the same study (164), but this is inconsistent across studies (133,144,165)

6.2.4 Exercise in combination with DMF for treatment of MS

An interesting finding in EAE showed that DMF in combination with exercise was associated with a greater clinical improvement and increased preservation of synaptic motor neuron input density in comparison to DMF alone or a comparator drug combined with exercise, indicating

a positive exercise DMT interaction (166). Corresponding studies on MS are yet to be conducted, although some existing studies have controlled for on-going DMT (e.g. see Kierkegaard *et al*. (158)). Whether the mode of action of exercise and DMF are complementary or overlapping has not been explored and was indirectly the subject of investigation in Paper IV. Prior studies have shown that both therapy modalities potentially mediate neuroprotective and immunomodulatory effects through redox signaling and activation of Nrf2 (90,95,167). Exercise also increases levels of D-β-hydroxybuturic acid, a ketone body synthesized from acetoacetate, which binds to the G-protein-coupled receptor HCAR2 common to DMF. In EAE, HCAR2 activation through DMF has been shown to reduce neurological deficits by decreasing neutrophil infiltration to the CNS and inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) in microglia, decreasing secretion of proinflammatory cytokines (93,94). Modulation of HIF-1a activity is also a common denominator for DMF and exercise although its regulation is dependent on exercise intensity and duration (95,168).

6.2.5 Exercise effects on healthy participants

The benefits of exercise for healthy participants have been extensively documented. The benefits span a wide range of functional systems, including neurological, cardiovascular and endocrinological. Regular exercise and physical activity decreases all-cause mortality within the observation period in a dose-response manner, which has been shown in multiple large-scale epidemiological studies (169–171). Immunologically, acute exercise bouts of less than 60 minutes increase circulating levels of neutrophils, $CD8^+$ T cells, NK cells and naïve B cells (172), thought to participate in a temporarily increased immune surveillance. Most commonly upregulated cytokines in association with exercise are IL-4, IL-6, IL-8, IL-10 and IL-12p40 (173). IL-6 is released directly from muscle tissue and participates in regulating inflammation through release of cortisol and blunting the TNF-α response. Exercise also gives rise to the release of other related proteins such as fibroblast growth factor (FGF)21 which regulates insulin sensitivity and glucose metabolism among several other functions (174).

6.2.6 Exercise and the kynurenine pathway

A potential exercise related marker for immunomodulation is the tryptophan metabolite kynurenic acid (KYNA) which is neuroprotective against excitotoxicity (175). Agudelo *et al*. have shown that exercise induces skeletal muscle peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) which through increased expression of kynurenine aminotransferases enhances the conversion of kynurenine to KYNA (176). This shift resulted in a protection from stress-induced changes associated with depression. Kynurenine and 3-hydroxykynurenine are also involved in glutamate metabolism and neuroinflammaton. Exercise has been shown to increase KYNA peripherally also in humans, though no studies have been conducted on MS patients (177).

7 THESIS AIMS

The overarching aim of the thesis was to determine the clinical effects of the MS drug DMF, explore its mode of action and compare it to that of physical exercise to identify possible common denominators.

Paper I

To evaluate the clinical effectiveness of DMF in comparison to other initial treatment options for patients with RRMS in Stockholm and Västerbotten counties and assess frequency and severity of adverse events.

Paper II

To evaluate clinical effectiveness of DMF in comparison to common initial and secondary treatment options for patients with RRMS with nationwide coverage and assess frequency and severity of adverse events.

Paper III

To characterize the effect of DMF on RRMS patient lymphocyte and monocyte counts, function and inflammatory mediators to distinguish patients responding to treatment with absence of clinical disease activity from non-responders.

Paper IV

To characterize the effect of aerobic exercise on inflammatory protein markers in plasma and CSF on healthy human participants.

8 METHODS

8.1 THE SWEDISH MS REGISTRY

The Swedish MS registry was launched in 2001 as part of the Swedish neuro registries that collects data on several neurological diseases including myasthenia gravis, Parkinson's disease and epilepsy. To date, the registry includes 20571 patients from all 21 counties in Sweden with a coverage rate almost 80% of prevalent MS cases (178). The data collected include patient characteristics, clinic visits, measures of clinical disease activity such as relapses and MRI lesions, therapies, functional scales, laboratory tests and are registered through an internet-based platform by physicians and nurses. The registry has served as the basis for more than 100 scientific reports spanning from epidemiological to neuroimmunological and genetic studies (178). A recent validation study from our group showed high validity for registered data (179). The registry was the primary data source for Paper II and was an important source for cross-referencing medical record data in Paper I.

8.2 THE KAPLAN-MEIER ESTIMATOR

The Kaplan-Meier estimator is a widely used univariate analysis that describe survival based on one predictive factor. In a clinical setting, these events are typically clinical signs of disease progression, discontinued treatment use or disease related death. Kaplan-Meier curves are frequently used to visualize these probabilities over a given time period (as in Paper I & II). Kaplan-Meier estimators are usually a univariate model, which means confounding factors such as age, sex and comorbidities are not adjusted for, which should be taken into account when interpreting results.

8.3 COX PROPORTIONAL HAZARDS REGRESSION

The Cox proportional hazards regression is the most common method for assessing the influence of predictor variables on survival rates in clinical studies. As with Kaplan-Meier estimations, it is often used for risk of treatment discontinuation (as in Paper I & II) and disease progression on treatment but can factor in multiple independent (predictor) variables to the outcomes. The model is based on the assumption that the effects of the predictor variables on an event are constant throughout the observation period and provides unreliable results when these conditions are not met.

8.4 PROPENSITY SCORE

The propensity score is a commonly used method in observational treatment studies with two or more treatment options. In absence of randomization to treatment, patients are often more likely to be assigned a certain treatment depending on a set of observed covariates, collectively termed confounding factors. The propensity score is utilized to factor in this in the assessment of study outcomes and is formulated as a probability ranging between 0 and 1. The propensity score can be used in several ways to control for confounding on effect size of treatment, such as propensity score matching and covariate adjustment using the propensity score. The latter is used in Paper I as an add on factor in hazard rate calculations on drug survival and clinical drug

efficacy measures. The benefits of the propensity score are limited by available clinical characteristics, favoring larger sets of variables (180).

8.5 PROXIMITY EXTENSION ASSAY

The proximity extension assay (PEA) uses a proximity extension technology to enable a high throughput multiplex proteomic immunoassay (181). The panel included in Paper III and IV contained 92 cytokines and chemokines and a selection of other immune-related proteins. The assay utilizes epitope-specific binding and hybridization of a set of paired antibodies linked to oligonucleotide probes, which subsequently can be amplified using a quantitative polymerase chain reaction to quantify relative protein concentrations. The method thus allows for both highly specific protein detection with small quantities of sample. Due to limited use, documented methodological shortcomings relative to other proteomic approaches are sparse (182).

9 RESULTS AND DISCUSSION

9.1 PAPER I

In this study, we conducted a retrospective observational cohort study on RRMS patients starting initial treatment in two Swedish counties: Stockholm and Västerbotten. RRMS patients in Stockholm County treated with DMF had similar rates of drug discontinuation as did interferons, fingolimod and natalizumab. While the main focus of the article came to be rituximab due to its superior effect on both decreasing relapse rate (hazard rate adjusted for propensity score (HR) for discontinuing DMF in comparison to RTX was 15.1 (95% confidence interval (CI) 3.9 – 58.0; see figure 4). Patients treated with DMF had a HR of 3.4 (95% CI 1.0 – 11.8) of having a relapse, HR of 8.4 (95% CI 1.7 – 72.1) of showing Gd^+ on MRI and roughly double the rate of adverse events (50% for DMF compared to 23.3% for RTX) during the study period which spanned little over three years.

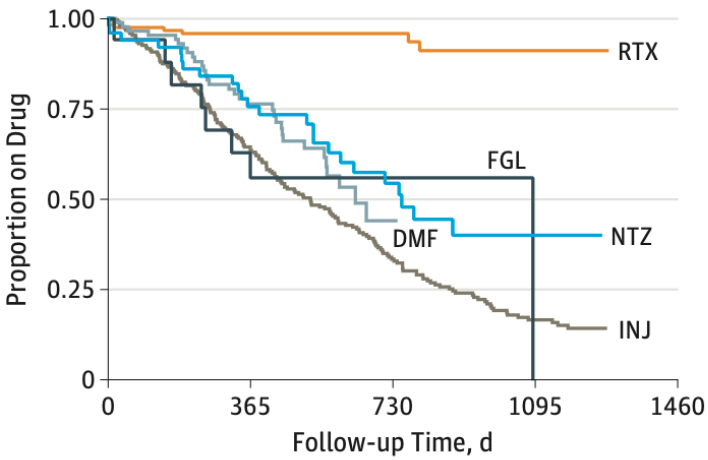

Figure 4. Kaplan-Meier curve of drug discontinuation of RRMS patients in Stockholm and Västerbotten counties. Patients treated with rituximab (RTX) had significantly lower rates of drug discontinuation compared to alternatives. Dimethyl fumarate (DMF), on the other hand, proved comparable to interferons and glatiramer acetate (INJ jointly), which is standard initial therapy for RRMS in many countries.

We next compared outcomes of RRMS patients treated in Stockholm and Västerbotten due to the then strikingly different treatment strategies applied in the two counties. In Stockholm, most patients were initially treated with common treatment choices for newly diagnosed RRMS, including interferons, glatiramer acetate and, in increasing rates, DMF. In Västerbotten County, however, the same category of patients was consistently initiated on treatment with RTX, thus allowing for a natural experiment comparing treatment strategies. Drug discontinuation, rate of clinical relapses, Gd^{+} MRI scans and adverse events were for all newly diagnosed patients in Västerbotten County similar to that of patients treated with RTX irrespective of county of residence, thereby partially mitigating potential selection bias with regards to selection of therapy (see figure 5).

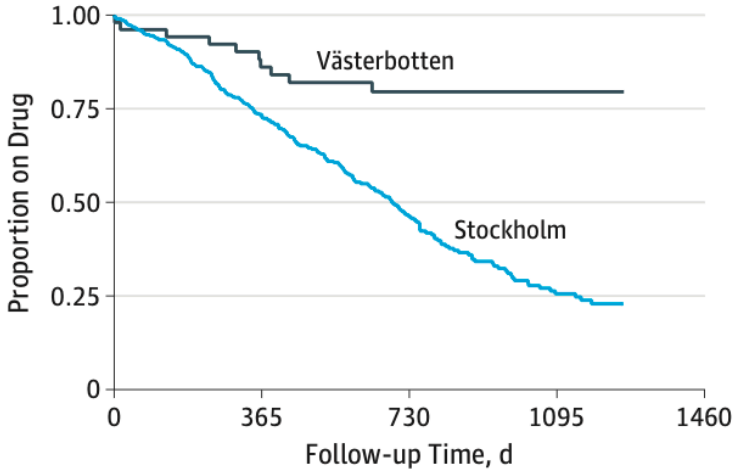

Figure 5. Kaplan-Meier curve of drug discontinuation of RRMS patients compared between Stockholm and Västerbotten counties irrespective of specific treatment. The treatment strategy in Västerbotten County consistently favored rituximab treatment for newly diagnosed RRMS patients, whereas Stockholm County generally used standard options interferons and glatiramer acetate. Drug persistence was superior in Västerbotten County.

The study provides class IV evidence for DMF being a safe and moderately effective initial therapy for RRMS patients. We identified several limiting factors, aside from the non-randomized study design that should be taken into consideration when interpreting results. Among others stated in the article were differing treatment follow-up guidelines, physician influence on therapy switching, lack of standardized procedures for registration of baseline data. Despite these shortcomings, similar outcomes for both RTX and DMF have been found in studies following Paper I.

9.2 PAPER II

While the comparative efficiency of DMF was assessed in comparison to RTX in Paper I, the clinical profile of DMF in comparison to interferons, glatiramer acetate and fingolimod was only partially investigated. This necessitated Paper II, where DMF was further characterized as both an initial and secondary treatment option in comparison standard choices for RRMS patients.

The second treatment cohort was collected retrospectively from the national internet-based MS registry (SMSreg; neuroreg.se), further described in the Methods section. As an initial treatment for RRMS, DMF had a superior clinical effect to interferons and glatiramer acetate, the most common choices for newly diagnosed patients in an escalation treatment strategy (see section 5). The rate of drug discontinuation was lower among DMF treated patients (HR 0.46, 95% CI 0.37 – 0.58; see figure 6), and had also a lower annual relapse rate (ARR; 0.04, 95% CI 0.03 – 0.06 compared to 0.10 95% CI 0.07 – 0.13) and time to first relapse was significantly longer ($p < 0.05$). As secondary therapy, DMF had a higher discontinuation rate than fingolimod (HR 1.51, CI 1.08 – 2.09, $p < 0.05$; see figure 7), but did not differ with regards to ARR (0.03, CI 0.02-0.05 vs 0.02, CI 0.01 – 0.04; $p = 0.41$) or time to first relapse ($p = 0.20$).

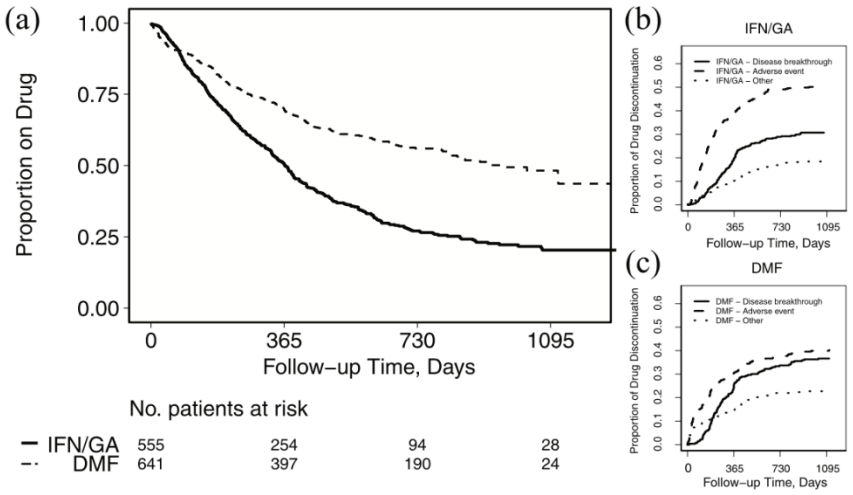

Figure 6. Initial treatment of RRMS patients with either dimethyl fumarate (DMF) or interferons/glatiramer acetate (IFN/GA). Kaplan-Meier curve of drug survival on a nationwide cohort (a). Treatment continuation of DMF was superior to IFN/GA over three years. Causes of discontinuation for the specific therapies shown in b and c. The most common cause of drug discontinuation was adverse events among both IFN/GA and DMF treated patients.

The most common cause of discontinuation for DMF as initial and secondary treatment was adverse events (17.2% and 20.8%, respectively) followed by disease breakthrough. Discontinuations due to unspecified reasons were high in both treatment groups including for comparator drugs. We speculated that this was in part driven by patients being placed on other more effective therapies such as rituximab. Assessing prescription rates for DMF and standard initial treatments interferons and glatiramer acetate, we found that they had decreased during the study period. Stratifying calendar year of prescription, however, did not influence discontinuation rates across the different therapies. In summary, Paper II suggests that DMF could benefit RRMS patients with a mild disease course as an initial therapy to a greater extent than interferons and glatiramer acetate. However, due to tolerability issues and the general shift towards early treatment with RTX in Sweden, clinical use of DMF is expected to remain limited with the current indication.

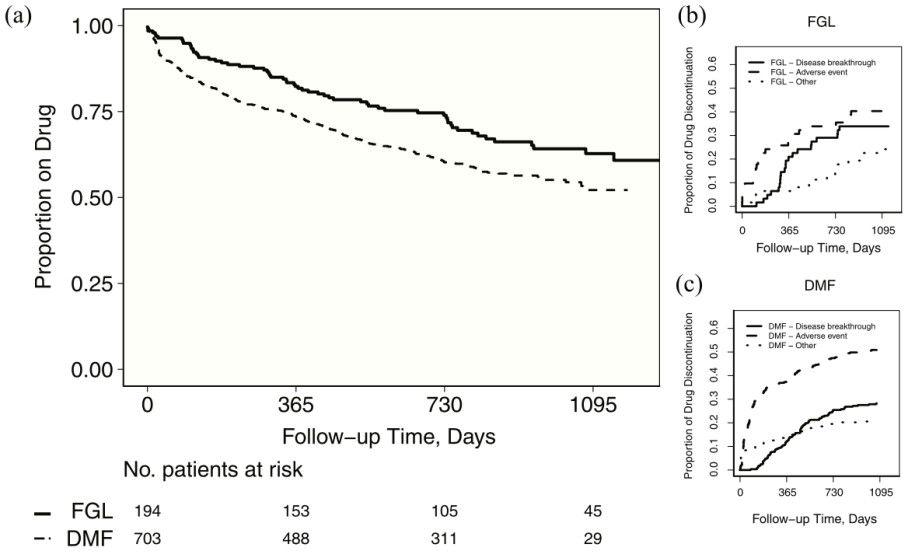

Figure 7. Secondary treatment of RRMS patients with either dimethyl fumarate (DMF) or fingolimod (FGL). Kaplan-Meier curve of drug survival on a nationwide cohort. Treatment continuation of DMF was inferior to FGL over three years despite no differences in effects on clinical disease activity (a). Causes of discontinuation for the specific therapies shown in b and c. For FGL, the most common cause of drug discontinuation was adverse events, as was the case for DMF.

9.3 PAPER III

In parallel with establishing the clinical treatment effect on RRMS patients in Paper I & II, our research group sought to characterize the underlying immunological mechanisms through which DMF exerts its effects in humans. Prior studies had shown DMF to confer an ameliorating effect in EAE rats by increasing the activity of the transcription factor Nrf2 which regulates the expression of several antioxidant proteins, thereby modulating the oxidative environment. Paper III thus focused on further exploring the effects of DMF on redox balance and redox signaling in the innate and adaptive immune system. Due to the low penetrance of DMF across the BBB, the main immunological effect was hypothesized to occur in blood, as part of the peripheral compartment.

Patients treated with DMF had increased circulating levels of the oxidative marker isoprostane 8.12-iso-iPF2α-VI at three- and six-months follow-up compared to baseline. As 8.12-iso-iPF2α-VI is generated through non-enzymatic oxidation, its increase reflects general shift to a more oxidative environment. This was further confirmed by comparing the transcriptional levels of differentially expressed mRNAs in $CD14^+$ monocytes at baseline and six months, which showed an enrichment in upregulation of genes involved in oxidative stress response.

To examine the mechanistic aspects of the DMF treatment effect, patients responding to treatment with absence of clinical signs of disease activity during an observation period of up to two years were distinguished from non-responders. Among responders, we observed higher levels of monocytes in blood after three months therapy persisting to 12 months. Lymphocyte counts were also lower at 12 months compared to baseline for responders (see figure 8). Monocyte ROS production assessed using dihydrorhodamine-123 in *in vitro* stimulation was higher

among responders, although general spontaneous ROS production was generally lower among DMF treated RRMS patients compared to untreated.

A SNP in NOX3 (minor *G* allele) displayed association with both lowered ROS generation in monocytes (0.057) and non-response to DMF treatment (p = 0.036), suggesting a possible direct link. Additionally, several SNP within NOX-producing complexes were associated with DMF treatment.

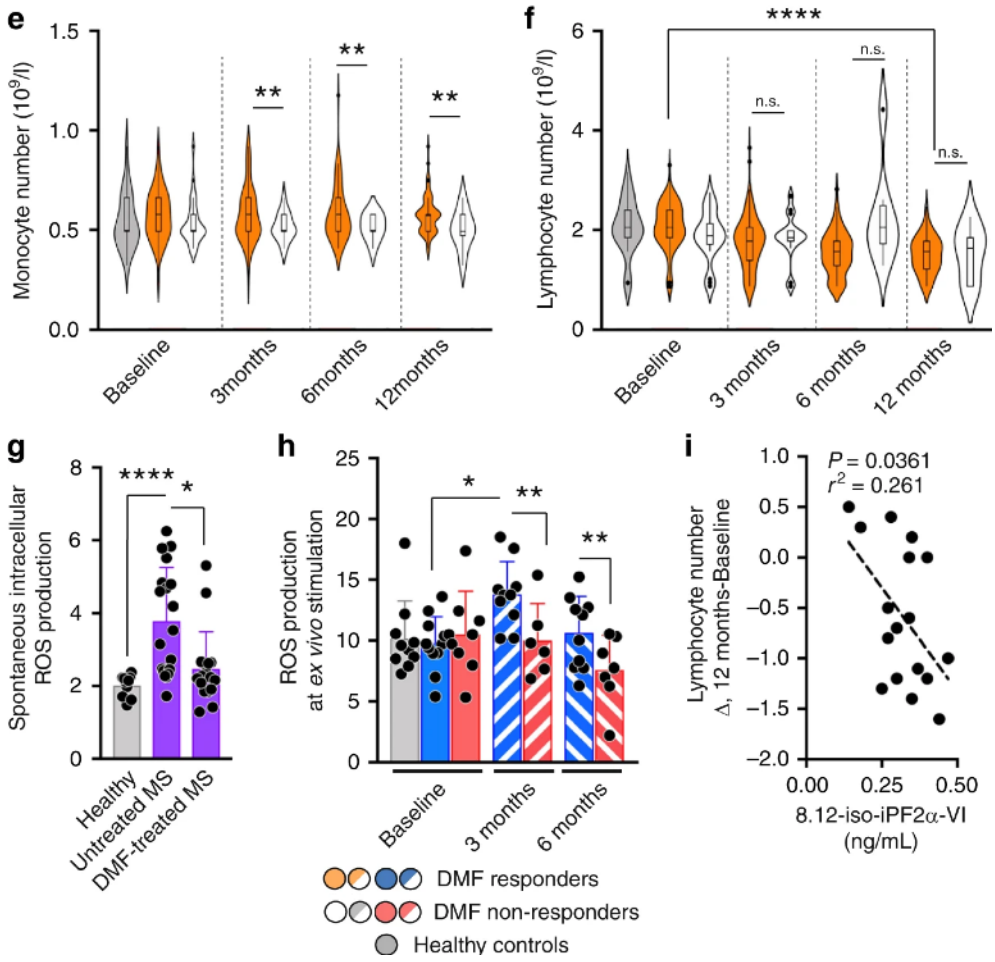

Figure 8. Monocyte and lymphocyte counts in blood along with monocyte ROS production. Monocyte counts increased in patients responding to DMF treatment with absence of clinical signs of MS disease activity at three, six and 12 months compared to non-responders(e). Lymphocyte counts decreased at 12 months among responders (f). Monocyte production of reactive oxygen species also increased among responders at three and six months (h).

DNA methylation changes in $CD14^+$ monocytes profiled with Illumina EPIC arrays were associated with regulation of apoptosis, metabolism and cell communication were observed at three months compared to baseline but had reverted back at six months. $CD4^+$ T cells, on the other hand, displayed methylation changes mostly between three and six months. Differentially methylated regions were associated with cell proliferation, apoptosis, migration and differentiation of T_h17 and T_{reg} cells.

DMF responders also had altered proportions and numbers of several subsets of $CD4^+$ T cells at six months. Responders had proportional increases but lowered number of naïve ($CD45RA^+$ $CCR7^+$) T cells, decreased proportion and numbers of T_{CM} ($CD45RA^-$ $CCR7^+$) cells and decreased proportions of T_{EM} ($CD45RA^-$ $CCR7^-$) cells.

Cytokine levels for responders were lower for IL-12B, IL-17C, CCL28 and CXCL9 at six months compared to baseline.

Collectively, these findings provide further support for the concept of monocyte ROS signaling being necessary for autoimmune regulation through regulation of T cells. Especially interesting is the possible direct genetical link between ROS generating capacity of monocytes and treatment response to DMF.

9.4 PAPER IV

Given the inconsistencies in prior studies with regard to cytokine/chemokine levels following exercise interventions, we opted to explore a wide array of immune protein markers with the multiplex assay Olink™ (further description in section 8.5).

In Paper IV we showed that healthy participants completing an intense aerobic exercise protocol for four days had upregulation of several immune protein markers when comparing follow-up and baseline samples from CSF and plasma. The effects from moderately intense aerobic exercise, which comprised the second group were less pronounced. See figure 9 for protein marker results.

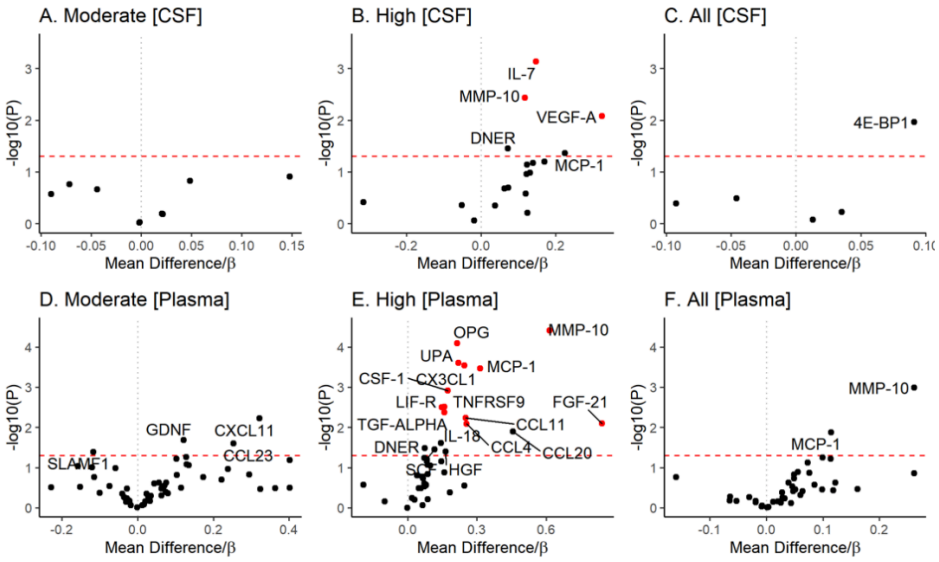

Figure 9. Inflammatory protein markers in cerebrospinal fluid (CSF) and plasma in healthy participants after highly or moderately intense aerobic exercise. Volcano plots summarize the mean difference and significance (P) from paired Student t-tests comparing baseline and follow-up levels of inflammation-related proteins after intervention with moderate (left) or high (middle) intensity exercise in CSF (first row), plasma (2nd row). Combined analyses with both intervention groups for determining correlation between proteins levels and intensity of exercise is shown (right). The red dashed line indicates an exploratory cutoff of p=0.05 and associations with P-false discovery rate <0.05 are highlighted in red.

Vascular endothelial growth factor (VEGF)-A and fibroblast growth factor (FGF)-21 were among the prior known exercise associated markers that increased post intervention. Surprisingly, common inflammatory cytokines such as IL-1, IL-6, TNF-α and IFN-γ did not occur in detectable levels either at baseline or follow-up, which may relate to technical issues with the detection platform.

Tryptophan metabolites were analyzed along with neurotransmitters glutamate, gamma-aminobutyric acid and serine using an ultra-performance liquid chromatography-tandem mass spectrometry system and high-performance liquid chromatography with fluorescence detection. CSF levels of kynurenic acid, picolinic acid and 3-hydroxykynurenine increased in the highly intense exercise group. Plasma levels of tryptophan and kynurenine decreased, in all approximating prior experimental findings in mice by Agudelo *et al*. (176). Interestingly, tryptophan metabolites and inflammatory protein markers showed correlation for many markers in both CSF and plasma, indicating a co-regulation of the two systems.

The findings in Paper IV underscored that importance of exercise intensity as a determinant for regulation of inflammatory protein markers. Relating to EAE studies, higher intensity of exercise also yields a greater inhibitory effect on disease severity, as seen in Fainstein *et al.* (148). High intensity exercise leads to cognitive benefits and modulation of MMP (see section 4.3) observed in RRMS patients, although study protocols have not been long enough (three weeks) to distinguish differences in clinical markers of RRMS disease activity (165). Of the RRMS exercise studies with extensive cytokine profiling, the study with the highest reported intensity also had the clearest discernible effect on inflammatory cytokines, including TNF-α, although findings in comparison to similar studies (but with slightly less intense protocols) are conflicting (133,158). The potential reasons why there relatively sparse overlap between studies are many, including duration of effect after exposure, dosing and training protocols, selection of sample medium (i.e. plasma, serum, CSF or tissue), choice of biomarkers and assay sensitivity.

Concerning the actual commonalities across exercise and DMF, as shown in Paper III and Paper IV, there was little overt overlap with regards to cytokine profiles in plasma or CSF. We also compared immune protein profiles as measured on the same Olink™ panels in plasma and CSF between DMF treated RRMS patients and RRMS patients participating in an exercise intervention from a separate and previously published cohort (158). Overlapping immune protein markers between groups were relatively few and inconsistent, as shown in table 1.

However, the main overlapping findings, as seen, include CCL4, FGF21, latency associated peptide (LAP)-TGF-β1 and TNF receptor super family 9 and will be further discussed with regards to their relevancy in an MS and exercise context.

Table 1. Comparison of differences in immune protein markers after dimethyl fumarate treatment and exercise in MS patients and aerobic exercise of moderate and high intensity in healthy participants.

Protein biomarker	MS DMF		Running, intense		Running, moderate		MS exercise	
	Plasma	CSF	Plasma	CSF	Plasma	CSF	Plasma	CSF
ADA		-						
CASP-8							-	
CCL4	+		+					
CCL11			+					
CD244							+	+
CD40		-					-	
CD5	-	-						
CDCP1	+	-						
CSF-1			+					
CST-5	-							
CX3CL1			+					+
CXCL1							-	
CXCL6							-	
CXCL9	+							
EN-RAGE	-						+	
FGF21	+		+					
FGF23	+							
FGF5		-						
FLT3L	+							-
IL-12B	+	-						
IL-17C	-							
IL-18R1		-						
IL-7				+			-	
LAP-TGF-B-1		+					-	+
LIF-R			+					
MCP-1			+					
MCP-2							-	
MCP-4							-	
MMP-1							-	
MMP-10			+	+				
OPG			+					
SIRT2							-	
ST1A1							-	
STAMPB							-	
TGF-A			+					
TNF-B		-						
TNFRSF14		-					-	
TNFRSF9	+	-	+					
TWEAK	-							
UPA			+					
VEGF-A				+				

+: increased levels after intervention. -: decreased levels after intervention.

CCL4, also known as macrophage inflammatory protein-1β, is a homing molecule for monocytes among other immune cells who also can produce CCL4, of which neutrophils, T cells, B cells and fibroblasts among other cells are capable. In MS, CCL4 is predominantly found in parenchymal and perivascular macrophages that contain myelin degradation products and is assumed to promote disease activity through further leucocyte recruitment (183). It is also an important factor for T cell adhesion to vascular walls, necessary for cell migration (184). CCL4 along with increases in IL-12 secretion from dendritic cells favor a T_h1 response for parasite

clearing (185). Increased CCL4 levels in serum in isolation is seen with antibody-mediated demyelinating diseases but not MS (186). In combination with circulating (plasma) markers human growth factor, CCL11 and epidermal growth factor, however, it can be used to distinguish RRMS from SPMS and PPMS (187). Levels of CCL4 are generally lower in plasma of SPMS and PPMS patients compared to RRMS. The degree of inflammatory disease activity was not specified for these patients, but it opens up for speculation as to whether it might signify lower inflammatory, cell recruiting activity. CCL4 readily diffuses across experimental endothelial monolayers, increasing vascular adhesion of activated $CD4^+$ T cells and memory T cells (188). As primarily monocytes and lymphocytes are mobilized in both DMF treatment and transiently during physical exercise, increased CCL4 signaling is to be expected. However, CCL4 was not differentially increased in treatment responders on DMF in Paper III.

TNF receptor super family 9, also named CD137, was increased in plasma of DMF and the intense running cohort but lowered in DMF CSF. CD137 functions as a costimulatory molecule on T cells. CD137 has been shown necessary for EAE development (189). Binding to CD137L, CD137 promotes IL-2 and -4 secretion and favor differentiation into $CD8^+$ T cells. Prior studies show CD137 increased in serum and CSF of RRMS patients (190). Comparing plasma CD137 levels in responders and non-responders to DMF treatment did not show significantly differing plasma levels of CD137.

LAP-TGF-β1 was found to be increased in CSF in DMF treated RRMS patients and exercised RRMS patients. As measuring of TGF-β1 levels directly associated with low reliability, the more stable precursor form non-covalently bound to LAP is often preferred. Upon cleaving of LAP, TGF is activated. Interestingly, ROS exposure rapidly activates latent complex bound TGF-β (191). Clinical research on LAP-TGF-β1 is sparse but it has been reported to be increased in RRMS patients receiving vitamin D-therapy, however, without any clear relation to effectiveness measures (192). Activated TGF-B facilitates remyelination, thus potentially of great benefit for MS patients (193). In EAE, TGF-β1 in combination with IL-6 promotes differentiation of $CD4^+$ T cells to Th17 cells (194). While the proinflammatory effect of the latter is consistent with described MS pathology, TGF-β1 serve beneficial functions. It is also one of two upregulated remyelination factors in the set of studies, drawing focus on the possible role of DMF in facilitating this. (For further investigation into this topic, see the thesis by Karl Carlström.)

The perhaps most interesting factor increased by both DMF and high intensity running in plasma is FGF21, even if it was not increased in exercised RRMS patients. In a separate, non-published analysis DMF treated RRMS patients had increased plasma levels of FGF21 by 14 percent compared to baseline. FGF21 is a well-studied cytokine mainly secreted by the liver and contracting muscles. It functions as an important mediator in glucose and lipid metabolism in response to physiological stressors such as exercise and fasting (195,196). Although high peripheral levels of FGF21 have been associated with several metabolic diseases including diabetes type II, administration of FGF21 analogs improve the blood lipid profile in humans and improves insulin signaling and provides resistance to weight gain from overfeeding in mice

(197). Treatment of patients with diabetes type II with FGF21 is under development. FGF21 has also been proposed as a regulator of oxidative stress as it increases the expression of anti-oxidative enzymes such as superoxide dismutase 2 (198). Increased activity of Nrf2 is associated with higher peripheral levels of FGF21 in mice (199). Interestingly, peripherally derived FGF21 readily crosses the BBB and promotes proliferation in oligodendrocyte progenitor cells and subsequent remyelination through binding to B-klotho (200,201).

In summary, though none of the differentially regulated biomarkers were consistent across all studies, the few overlapping protein markers highlight possible common mechanisms between exercise and DMF. Most prominent, perhaps, is FGF21 as a potential target for induction by DMF treatment and exercise, which could benefit neurological recovery in MS patients after manifest myelin and oligodendrocyte damage. This adds a second mechanism by which DMF treatment (and exercise) might benefit relapse recovery in MS, as Nrf2 increases expression of glutathione S-transferase 4α, which has been shown to benefit oligodendrocyte differentiation and remyelination (202). Beneficial effects from FGF21 also seem to pertain to diabetes type II, a common MS comorbidity as described in section 4.5.

10 CONCLUSIONS AND FUTURE PERSPECTIVES

Following the four studies included in the thesis several conclusions on DMF in RRMS can be drawn:

I) DMF is a moderately effective therapy in comparison to current treatment alternatives for RRMS patients both as initial and secondary therapy.

II) The mode of action of DMF is partially related to monocyte counts and monocyte ROS production in interaction with lymphocytes in ways that potentially could be utilized in a clinical setting to distinguish patients responding to treatment from non-responders.

III) Despite several mechanisms of DMF treatment overlap with that of intense aerobic exercise, there are few and inconsistent common denominators with regard to immune marker proteins.

Further queries remain as to how DMF (and in extension pharmacological treatment) and exercise complement each other clinically, and if there are opportunities for synergistic effects on disease modulation and disease-related functional impairments. As exercise is shown beneficial for several MS symptoms and common comorbidities and proven safe, clinical trials in combination with DMF and other DMTs would be both interesting from a research perspective and immediately useful for participating MS patients. Efforts to integrate exercise in MS treatment are already ongoing, as described in section 6. As clinical effects are likely to require intervention periods of up to a year given adequate sample size, disease markers such as NFL, cell populations, cytokine profiles or epigenetic changes in immune cell subsets could be of value and require shorter interventions.

Traditional RCTs are considered the gold standard for determining the risk-benefit of drugs. However, exceedingly large studies would be needed to compare across a number of therapies and different subpopulations of MS patients. In addition, the interest of doing such studies by the pharmaceutical industry is low and most patients are also unwilling to participate in trials with randomized treatments. Instead it is an important task for health authorities and academic research to device pragmatic studies in large real-world populations that collect multi-modal and detailed information on relevant effectiveness and adverse outcomes. This can be exemplified by the on-going prospective collection of data on patient treated with RTX and other treatment choices in COMBAT-MS, an observational drug trial funded by a federal US academic grant, comprising 3500 patients across all of Sweden. As more MS treatments are about to be introduced to market, evaluating the relative risk-benefit in an expedient manner is of great importance in order to improve both patient outcomes and cost-benefit. DMF, for example, has two recently approved alternatives in monomethyl fumarate (Bafiertam™) and diroximel fumarate (Vumerity™). Both aim to mitigate the tolerability issues of DMF, potentially making them more useful for newly diagnosed patients with a mild disease course.

Despite much research is going into developing methods for effective drug repurposing, the serendipitous discovery of the benefits of DMF and RTX for MS highlight the inefficiencies

of this practice. Considering the clinical efficacy of RTX, earlier discovery would have been beneficial for MS patients on a large scale. With social security numbers and extensive and combinable registries on public health, Sweden is well-set for being the base for new such discoveries. Continued cross-disciplinary research and collaboration across clinics are likely to be key going forward in this endeavor.

Further mechanistic studies are warranted to define the mechanism(s) of action of DMF so as to establish drug targets that are more specific. Research from our group has already partially explored this venue (203). With increased availability and utility of multi-omics (with its latest addition of the microbiome), as well as higher cellular resolution through single-cell sequencing, investigative studies on human samples are likely to yield more useful data. Handling and integrating data sets of this size require tools that are both in use and in rapid development, such as machine learning algorithms that can assess outcome relevant correlations from complex data.

11 SIGNIFICANCE

With increasing treatment options for RRMS patients, one of the main current challenges is to assess their comparative benefits, as well as identifying treatments that not only reduce inflammatory activity but also improve function and structural integrity of the CNS. Both DMF and rituximab represent alternatives that are superior in effectiveness to previously existing initial treatment choices for RRMS and a shift towards increasing early use of these therapies will be important to preserve neurological functions among newly diagnosed patients. Nonetheless, it is clear that they display different modes of action and further research will be needed to identify potential markers of beneficial treatment responses, including long term safety, in order to optimize drug tailoring and risk-benefit at the individual level. For manifest neurological deficits, however, current DMTs have no proven effect, whereas accumulating evidence shows exercise to beneficial for a range of debilitating symptoms, including fatigue and motor deficits. Comorbidity, which adds additional burden of disease and may also worsen MS disease status, can also be alleviated through regular physical exercise. Despite this, the clinical use of exercise as a supplement to medical therapies is still scarce, likely as a consequence of still limited high quality studies, restricted mechanistic understanding of the effects and optimized training protocols for individual needs (121). Exercise intensity, for example, seems to have differential immunomodulatory benefits which is further explored within Paper IV. Potential common denominators between DMTs and exercise on intrathecal inflammatory markers could also lead to better understanding of processes involved in the neurodegenerative aspects of the disease that are beyond most current drug therapies. In summary, combining the two approaches represented in this thesis work, namely optimizing early DMT and engaging in regular physical activity together with other life-style modifications likely improves chances of beneficial long-term outcomes. The lack of obviously and consistently overlapping molecular pathways between DMF and physical activity when compared with protein markers suggests that one cannot substitute for the other and that there is an added benefit of combining them. For patients, the benefits are likely to be manifold both in the long and short term, not only delaying MS disease progression, but also reducing the risk of comorbidity. Together this can help our patients preserve as much of their full potential in life.

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13 REFERENCES

1. Compston A, Coles A. Multiple sclerosis. Lancet [Internet]. 2008;372(9648):1502–17. Available from: http://dx.doi.org/10.1016/S0140-6736(08)61620-7
2. Ahlgren C, Odén A, Lycke J. High nationwide incidence of multiple sclerosis in Sweden. PLoS One. 2014;9(9).
3. Wallin MT, Culpepper WJ, Nichols E, Bhutta ZA, Gebrehiwot TT, Hay SI, et al. Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol [Internet]. 2019 Mar;18(3):269–85. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1474442218304435
4. Confavreux C, Vukusic S. Natural history of multiple sclerosis: A unifying concept. Brain. 2006;129(3):606–16.
5. Ragonese P, Aridon P, Salemi G, D'Amelio M, Savettieri G. Mortality in multiple sclerosis: A review. Eur J Neurol. 2008;15(2):123–7.
6. Scalfari A, Knappertz V, Cutter G, Goodin DS, Ashton R, Ebers GC. Mortality in patients with multiple sclerosis. Neurology. 2013;81(2):184–92.
7. Goodin DS, Reder AT, Ebers GC, Cutter G, Kremenchutzky M, Oger J, et al. Survival in MS A randomized cohort study 21 years after the start of the pivotal IFNβ-1b trial. Neurology. 2012;78(17):1315–22.
8. Manouchehrinia A, Beiki O, Hillert J. Clinical course of multiple sclerosis: A nationwide cohort study. Mult Scler. 2017;23(11):1488–95.
9. Simpson S, Wang W, Otahal P, Blizzard L, Van Der Mei IAF, Taylor B V. Latitude continues to be significantly associated with the prevalence of multiple sclerosis: An updated meta-analysis. J Neurol Neurosurg Psychiatry. 2019;90(11):1193–200.
10. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol [Internet]. 2010;9(5):520–32. Available from: http://dx.doi.org/10.1016/S1474-4422(10)70064-8
11. Grant WB, Mascitelli L. Evidence that the north-south gradient of multiple sclerosis may not have disappeared. J Neurol Sci [Internet]. 2012;315(1–2):178–9. Available from: http://dx.doi.org/10.1016/j.jns.2012.01.002
12. Lucas RM, Ponsonby AL, Valery P, Taylor B. Sun exposure and vitamin d are independent risk factors for cns demyelination. Neurology. 2011;77(14):1405–6.
13. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology [Internet]. 2004 Jan 13;62(1):60–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14718698
14. Runia TF, Hop WCJ, De Rijke YB, Buljevac D, Hintzen RQ. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. Neurology. 2012;79(3):261–6.
15. Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch H-M, Cheung R, et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. Neurology [Internet]. 2010 Jun 8;74(23):1852–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20427749
16. O'Connell K, Sulaimani J, Basdeo SA, Kinsella K, Jordan S, Kenny O, et al. Effects of vitamin D 3 in clinically isolated syndrome and healthy control participants: A double-blind randomised controlled trial . Mult Scler J – Exp Transl Clin. 2017;3(3):205521731772729.
17. Spelman T, Gray O, Trojano M, Petersen T, Izquierdo G, Hupperts R, et al. Seasonal variation of relapse rate in multiple sclerosis is latitude dependent. Ann Neurol. 2014;76(6):880–90.
18. Farez MF, Mascanfroni ID, Méndez-Huergo SP, Yeste A, Murugaiyan G, Garo LP, et

al. Melatonin Contributes to the Seasonality of Multiple Sclerosis Relapses. Cell. 2015;162(6):1338–52.
19. Hammond SR, English DR, McLeod JG. The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain [Internet]. 2000 May;123 (Pt 5(5):968–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10775541
20. Hedström AK, Sundqvist E, Bäärnhielm M, Nordin N, Hillert J, Kockum I, et al. Smoking and two human leukocyte antigen genes interact to increase the risk for multiple sclerosis. Brain. 2011;134(3):653–64.
21. Hedström AK, Åkerstedt T, Olsson T, Alfredsson L. Shift work influences multiple sclerosis risk. Mult Scler J. 2015;21(9):1195–9.
22. Hedström AK, Olsson T, Alfredsson L. High body mass index before age 20 is associated with increased risk for multiple sclerosis in both men and women. Mult Scler J. 2012;18(9):1334–6.
23. Hedström AK, Bomfim IL, Barcellos L, Gianfrancesco M, Schaefer C, Kockum I, et al. Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis. Neurology. 2014;82(10):865–72.
24. Hedström AK, Bäärnhielm M, Olsson T, Alfredsson L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. Mult Scler J. 2011;17(7):788–93.
25. Schwarz S, Leweling H. Multiple sclerosis and nutrition. Mult Scler [Internet]. 2005 Feb;11(1):24–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15732263
26. Westerlind H, Ramanujam R, Uvehag D, Kuja-Halkola R, Boman M, Bottai M, et al. Modest familial risks for multiple sclerosis: A registry-based study of the population of Sweden. Brain. 2014;137(3):770–8.
27. International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med [Internet]. 2007 Aug 30;357(9):851–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17660530
28. Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kemppinen A, Cotsapas C, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet. 2013;45(11):1353–62.
29. Costantino CM, Baecher-allan C, Hafler DA. Multiple Sclerosis and Regulatory T Cells. J Clin Immunol. 2008;28:697–706.
30. Frischer JM, Bramow S, Dal-bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009;132:1175–89.
31. Martin MD, Badovinac VP. Defining Memory CD8 T Cell. Front Immunol. 2018;9(November):1–10.
32. Moen SM, Simonsen CS, Flemmen HØ, Lauritzen T, Celius EG. The diagnostic value of IgG index versus oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis. Mult Scler J - Exp Transl Clin. 2020;6(1).
33. Willis SN, Stathopoulos P, Chastre A, Compton SD, Connor KCO. Investigating the Antigen Specificity of Multiple Sclerosis Central Nervous System-Derived Immunoglobulins. Front Neurol. 2015;6(November):1–9.
34. Li R, Patterson KR, Bar-Or A. Reassessing B cell contributions in multiple sclerosis. Nat Immunol [Internet]. 2018;19(7):696–707. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29925992
35. Jelcic I, Al Nimer F, Wang J, Lentsch V, Planas R, Jelcic I, et al. Memory B Cells Activate Brain-Homing, Autoreactive CD4+ T Cells in Multiple Sclerosis. Cell. 2018;175(1):85-100.e23.
36. Alvarez E, Piccio L, Mikesell RJ, Klawiter EC, Parks BJ, Naismith RT, et al. CXCL13

is a biomarker of inflammation in multiple sclerosis, neuromyelitis optica, and other neurological conditions. Mult Scler J. 2013;19(9):1204–8.
37. Alvarez E, Piccio L, Mikesell RJ, Trinkaus K, Parks BJ, Naismith RT, et al. Predicting optimal response to B-cell depletion with rituximab in multiple sclerosis using CXCL13 index , magnetic resonance imaging and clinical measures. Mult Scler J - Exp Transl Clin. 2015;1:1–9.
38. Hultqvist M, Olofsson P, Holmberg J, Bäckström BT, Tordsson J, Holmdahl R. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. Proc Natl Acad Sci U S A. 2004;101(34):12646–51.
39. Popescu BFG, Lucchinetti CF. Pathology of Demyelinating Diseases. Annu Rev Pathol Mech Dis. 2012;7(1):185–217.
40. Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, et al. Monocyte/Macrophage Differentiation in Early Multiple Sclerosis Lesions Materials and Methods Biopsy Material and Clinical Data. Ann Neurol. 1995;38:788–96.
41. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol [Internet]. 2000 Jun;47(6):707–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10852536
42. Smith KJ, Kapoor R, Felts PA. Demyelination : The Role of Reactive Oxygen and Nitrogen Species. Brain Pathol. 1999;92:69–92.
43. McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD. Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyefitis. J Exp Med. 1995;182(1):75–85.
44. Olson JK, Ercolini AM, Miller SD. A virus-induced molecular mimicry model of multiple sclerosis. Curr Top Microbiol Immunol. 2005;296(2):39–53.
45. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science [Internet]. 1996 Jun 28;272(5270):1947–50. Available from: http://link.springer.com/10.1007/s10898-013-0098-2
46. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol [Internet]. 2004 Apr;14(2):164–74. Available from: http://dx.doi.org/10.1038/nri3871
47. Ganter P, Prince C, Esiri MM. Spinal cord axonal loss in multiple sclerosis: A post-mortem study. Neuropathol Appl Neurobiol. 1999;25(6):459–67.
48. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci [Internet]. 2014 Jan;17(1):131–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24316888
49. Shah A. Fatigue in Multiple Sclerosis. Phys Med Rehabil Clin N Am [Internet]. 2009;20(2):363–72. Available from: http://dx.doi.org/10.1016/j.pmr.2008.12.003
50. Mills RJ, Young CA. A medical definition of fatigue in multiple sclerosis. QJM [Internet]. 2008 Jan;101(1):49–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18194977
51. Motl RW, McAuley E, Wynn D, Suh Y, Weikert M. Effects of change in fatigue and depression on physical activity over time in relapsing-remitting multiple sclerosis. Psychol Heal Med. 2011;16(1):1–11.
52. Motl RW, McAuley E. Symptom cluster and quality of life: Preliminary evidence in multiple sclerosis. J Neurosci Nurs. 2010;42(4):212–6.
53. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol. 2011 Feb;69(2):292–302.

54. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol [Internet]. 2018;17(2):162–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29275977
55. Filippi M, Rocca MA, Ciccarelli O, De Stefano N, Evangelou N, Kappos L, et al. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. Lancet Neurol [Internet]. 2016 Mar;15(3):292–303. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0031938416312148
56. Brex PA, Ciccarelli O, O'Riordan JI, Sailer M, Thompson AJ, Miller DH. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. N Engl J Med. 2002;346(3):158–64.
57. Jacobs LD, Beck RW, Simon JH, Kinkel RP, Brownscheidle CM, Murray TJ, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. N Engl J Med [Internet]. 2000 Sep 28;343(13):898–904. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11006365
58. Comi G, Filippi M, Barkhof F, Durelli L, Edan G, Fernández O, et al. Effect of early interferon treatment on conversion to definite multiple sclerosis: A randomised study. Lancet. 2001;357(9268):1576–82.
59. Okuda DT, Mowry EM, Beheshtian A, Waubant E, Baranzini SE, Goodin DS, et al. Incidental MRI anomalies suggestive of multiple sclerosis The radiologically isolated syndrome [Internet]. 2009 [cited 2020 Jul 13]. Available from: www.neurology.org.
60. Okuda DT, Siva A, Kantarci O, Inglese M, Katz I, Tutuncu M, et al. Radiologically Isolated Syndrome: 5-Year Risk for an Initial Clinical Event. Jacobson S, editor. PLoS One [Internet]. 2014 Mar 5 [cited 2020 Jul 13];9(3):e90509. Available from: https://dx.plos.org/10.1371/journal.pone.0090509
61. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: The 2013 revisions. Neurology. 2014;83(3):278–86.
62. Miller D, Compston A. The differential diagnosis of multiple sclerosis. McAlpine's Mult Scler Fourth Ed. 2005;71(suppl II):389–437.
63. Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: A potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry [Internet]. 1998 [cited 2020 Jul 13];64(3):402–4. Available from: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/9527161/
64. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017;89(22):2230–7.
65. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol [Internet]. 2017 Jun 1 [cited 2020 Jul 13];81(6):857–70. Available from: https://onlinelibrary-wiley-com.proxy.kib.ki.se/doi/full/10.1002/ana.24954
66. Salzer J, Svenningsson A, Sundströ P. Neurofilament light as a prognostic marker in multiple sclerosis.
67. Fainardi E, Castellazzi M, Bellini T, Manfrinato MC, Baldi E, Casetta I, et al. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. 2006;(March 2005):294–301.
68. Vogt MHJ, Lopatinskaya L, Smits M, Polman CH. Elevated Osteopontin Levels in Active Relapsing-Remitting Multiple Sclerosis. Ann Neurol. 2003;53:819–22.
69. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology [Internet]. 1983 Nov;33(11):1444–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6685237

70. Riahi F, Zijdenbos A, Narayanan S, Arnold D, Francis G, Antel J, et al. Improved correlation between scores on the expanded disability status scale and cerebral lesion load in relapsing – remitting multiple sclerosis Results of the application of new imaging methods. Brain. 1998;121:1305–12.
71. Popescu V, Agosta F, Hulst HE, Sluimer IC, Knol DL, Sormani MP, et al. Brain atrophy and lesion load predict long term disability in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2013;84(10):1082–91.
72. Marrie RA, Horwitz RI, Cutter G, Tyry T, Vollmer T. Association between comorbidity and clinical characteristics of MS. Acta Neurol Scand. 2011;124(2):135–41.
73. Munger KL, Chitnis T, Ascherio A. Body size and risk of MS in two cohorts of US women. Neurology [Internet]. 2009 Nov 10;73(19):1543–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19901245
74. Marrie RA. Comorbidity in multiple sclerosis: Past, present and future. Clin Invest Med. 2019;42(1):E5–12.
75. Piehl F. A changing treatment landscape for multiple sclerosis: Challenges and opportunities. J Intern Med. 2014;275(4):364–81.
76. Tur C, Kalincik T, Oh J, Sormani MP, Tintoré M, Butzkueven H, et al. Head-to-head drug comparisons in multiple sclerosis: Urgent action needed. Neurology. 2019;93(18):793–809.
77. de Jong HJI, Kingwell E, Shirani A, Cohen Tervaert JW, Hupperts R, Zhao Y, et al. Evaluating the safety of β-interferons in MS. Neurology [Internet]. 2017 Jun 13;88(24):2310–20. Available from: http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000004620
78. Ford C, Goodman AD, Johnson K, Kachuck N, Lindsey JW, Lisak R, et al. Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: Results from the 15-year analysis of the US prospective open-label study of glatiramer acetate. Mult Scler. 2010;16(3):342–50.
79. Ontaneda D, Tallantyre E, Kalincik T, Planchon SM, Evangelou N. Early highly effective versus escalation treatment approaches in relapsing multiple sclerosis. Lancet Neurol [Internet]. 2019;18(10):973–80. Available from: http://dx.doi.org/10.1016/S1474-4422(19)30151-6
80. Stankiewicz JM, Weiner HL. An argument for broad use of high efficacy treatments in early multiple sclerosis. Neurol Neuroimmunol Neuroinflamm. 2020;7:636.
81. Scalfari A, Neuhaus A, Daumer M, Muraro PA, Ebers GC. Onset of secondary progressive phase and long-term evolution of multiple sclerosis. J Neurol Neurosurg Psychiatry. 2014;85(1):67–75.
82. Beiki O, Frumento P, Bottai M, Manouchehrinia A, Hillert J. Changes in the risk of reaching multiple sclerosis disability milestones in recent decades: A nationwide population-based cohort study in sweden. JAMA Neurol. 2019;76(6):665–71.
83. He A, Merkel B, Brown JWL, Zhovits Ryerson L, Kister I, Malpas CB, et al. Timing of high-efficacy therapy for multiple sclerosis: a retrospective observational cohort study. Lancet Neurol [Internet]. 2020;19(4):307–16. Available from: http://dx.doi.org/10.1016/S1474-4422(20)30067-3
84. Honce JM, Nair K V., Sillau S, Valdez B, Miravalle A, Alvarez E, et al. Rituximab vs placebo induction prior to glatiramer acetate monotherapy in multiple sclerosis. Neurology. 2019;92(7):E723–32.
85. Atkins HL, Bowman M, Allan D, Anstee G, Arnold DL, Bar-Or A, et al. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. Lancet [Internet]. 2016;388(10044):576–85. Available from: http://dx.doi.org/10.1016/S0140-6736(16)30169-6

86. Schimrigk S, Brune N, Hellwig K, Lukas C, Bellenberg B, Rieks M, et al. Oral fumaric acid esters for the treatment of active multiple sclerosis: An open-label, baseline-controlled pilot study. Eur J Neurol. 2006;13(6):604–10.
87. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmaj K, et al. Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis. N Engl J Med [Internet]. 2012;367(12):1098–107. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1114287
88. Fox RJ, Miller DH, Phillips JT, Hutchinson M, Havrdova E, Kita M, et al. Placebo-Controlled Phase 3 Study of Oral BG-12 or Glatiramer in Multiple Sclerosis. N Engl J Med [Internet]. 2012;367(12):1087–97. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1206328
89. Gold R, Arnold DL, Bar-Or A, Hutchinson M, Kappos L, Havrdova E, et al. Long-term effects of delayed-release dimethyl fumarate in multiple sclerosis: Interim analysis of ENDORSE, a randomized extension study. Mult Scler. 2017;23(2):253–65.
90. Linker RA, Lee DH, Ryan S, Van Dam AM, Conrad R, Bista P, et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain. 2011;134(3):678–92.
91. Ballmann C, Mcginnis G, Peters B, Slivka D, Cuddy J, Hailes W, et al. Exercise-induced oxidative stress and hypoxic exercise recovery. Eur J Appl Physiol Occup Physiol. 2014;114(4):725–33.
92. Palmer AM. Multiple sclerosis and the blood-central nervous system barrier. Cardiovasc Psychiatry Neurol [Internet]. 2013;2013(1 SUPPL.):530356. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23401746
93. Chen H, Assmann JC, Krenz A, Rahman M, Grimm M, Karsten CM, et al. Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. J Clin Invest. 2014;124(5):2188–92.
94. Parodi B, Rossi S, Morando S, Cordano C, Bragoni A, Motta C, et al. Fumarates modulate microglia activation through a novel HCAR2 signaling pathway and rescue synaptic dysregulation in inflamed CNS. Acta Neuropathol. 2015;130(2):279–95.
95. Carlström KE, Ewing E, Granqvist M, Gyllenberg A, Aeinehband S, Enoksson SL, et al. Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis associates with ROS pathway in monocytes. Nat Commun [Internet]. 2019;10(1):3081. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31300673
96. Longbrake EE, Ramsbottom MJ, Cantoni C, Ghezzi L, Cross AH, Piccio L. Dimethyl fumarate selectively reduces memory T cells in multiple sclerosis patients. Mult Scler. 2016;22(8):1061–70.
97. Wu Q, Wang Q, Mao G, Dowling CA, Lundy SK, Mao-Draayer Y. Dimethyl Fumarate Selectively Reduces Memory T Cells and Shifts the Balance between Th1/Th17 and Th2 in Multiple Sclerosis Patients. J Immunol. 2017;198(8):3069–80.
98. Lückel C, Picard F, Raifer H, Campos Carrascosa L, Guralnik A, Zhang Y, et al. IL-17 + CD8 + T cell suppression by dimethyl fumarate associates with clinical response in multiple sclerosis. Nat Commun. 2019;10(1):5722.
99. Treumer F, Zhu K, Gläser R, Mrowietz U. Dimethylfumarate Is a Potent Inducer of Apoptosis in Human T Cells. J Invest Dermatol. 2003;121(6):1383–8.
100. Kornberg MD, Bhargava P, Kim PM, Putluri V, Snowman AM, Putluri N, et al. Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. Science (80-) [Internet]. 2018 Apr 27;360(6387):449–53. Available from: http://www.sciencemag.org/lookup/doi/10.1126/science.aan4665
101. Rostami-Yazdi M, Clement B, Schmidt TJ, Schinor D, Mrowietz U. Detection of metabolites of fumaric acid esters in human urine: implications for their mode of action. J Invest Dermatol. 2009;129(1):231–4.
102. Hadzic T, Li L, Cheng N, Walsh SA, Spitz DR, Knudson CM. The role of low

molecular weight thiols in T lymphocyte proliferation and IL-2 secretion. J Immunol [Internet]. 2005 Dec 15;175(12):7965–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16339532
103. Yadav SK, Soin D, Ito K, Dhib-jalbut S. Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis. 2019;463–72.
104. Hanson J, Gille A, Offermanns S. Pharmacology & Therapeutics Role of HCA 2 (GPR109A) in nicotinic acid and fumaric acid ester-induced effects on the skin OH. Pharmacol Ther [Internet]. 2012;136(1):1–7. Available from: http://dx.doi.org/10.1016/j.pharmthera.2012.06.003
105. Yukitake M. Drug-induced progressive multifocal leukoencephalopathy in multiple sclerosis: A comprehensive review. Clin Exp Neuroimmunol. 2018;9:37–47.
106. Gorelik L, Lerner M, Bixler S, Crossman M, Schlain B, Simon K, et al. Anti-JC Virus Antibodies : Implications for PML Risk Stratification. Ann Neurol. 2010;68(295):295–303.
107. Ruggieri S, Logoteta A, Martini G, Bozzao A, De Giglio L. *Listeria monocytogenes* – Induced Rhombencephalitis in a Patient With Multiple Sclerosis Treated With Dimethyl Fumarate. JAMA Neurol [Internet]. 2018;(D):2–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29610843%0Ahttp://archneur.jamanetwork.com/article.aspx?doi=10.1001/jamaneurol.2018.0368
108. Gajra A, Vajpayee N, Grethlein SJ. Response of myasthenia gravis to rituximab in a patient with non-Hodgkin lymphoma. Am J Hematol [Internet]. 2004 Oct;77(2):196–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15389902
109. Jacob A, Weinshenker BG, Violich I, McLinskey N, Krupp L, Fox RJ, et al. Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients. Arch Neurol [Internet]. 2008 Nov;65(11):1443–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18779415
110. Sveinsson O, Granqvist M, Forslin Y, Blennow K, Zetterberg H, Piehl F. Successful combined targeting of B- and plasma cells in treatment refractory anti-NMDAR encephalitis. J Neuroimmunol [Internet]. 2017;312:15–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28886955
111. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. N Engl J Med. 2017;376(3):221–34.
112. Smith MR. Rituximab (monoclonal anti-CD20 antibody): Mechanisms of action and resistance. Oncogene [Internet]. 2003 Oct 20 [cited 2020 Jul 13];22(47):7359–68. Available from: https://pubmed-ncbi-nlm-nih-gov.proxy.kib.ki.se/14576843/
113. Handel AE, Williamson AJ, Disanto G, Handunnetthi L, Giovannoni G, Ramagopalan S V. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. PLoS One. 2010;5(9):1–5.
114. Palanichamy A, Jahn S, Nickles D, Derstine M, Abounasr A, Hauser SL, et al. Rituximab Efficiently Depletes Increased CD20-Expressing T Cells in Multiple Sclerosis Patients. J Immunol. 2014;193(2):580–6.
115. Barr TA, Shen P, Brown S, Lampropoulou V, Roch T, Lawrie S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. J Exp Med. 2012;209(5):1001–10.
116. Luna G, Alping P, Burman J, Fink K, Fogdell-Hahn A, Gunnarsson M, et al. Infection Risks among Patients with Multiple Sclerosis Treated with Fingolimod, Natalizumab, Rituximab, and Injectable Therapies. JAMA Neurol. 2020;77(2):184–91.
117. Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue EW, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. N Engl J Med. 2006;354(9):911–23.
118. Carson KR, Evens AM, Richey EA, Habermann TM, Focosi D, Seymour JF, et al.

Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients : a report of 57 cases from the Research on Adverse Drug Events and Reports project. Blood. 2009;113(20):4834–40.
119. MCALPINE D. Multiple sclerosis: a plea for a fresh outlook. Br Med J [Internet]. 1957 Mar 2;1(5017):475–80. Available from: http://www.bmj.com.lcproxy.shu.ac.uk/content/bmj/1/5017/475.full.pdf
120. GEISLER WO, JOUSSE AT. Rehabilitation in disseminated sclerosis: an evaluation of 28 patients. Can Med Assoc J [Internet]. 1963 Jan 26;88(9):189–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13947028
121. Motl RW, Sandroff BM, Kwakkel G, Dalgas U, Feinstein A, Heesen C, et al. Exercise in patients with multiple sclerosis. Lancet Neurol [Internet]. 2017;16(10):848–56. Available from: http://dx.doi.org/10.1016/S1474-4422(17)30281-8
122. Motl RW, McAuley E, Snook EM. Physical activity and multiple sclerosis: a meta-analysis. Mult Scler [Internet]. 2005 Aug;11(4):459–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16042230
123. Johansson S, Ytterberg C, Gottberg K, Holmqvist LW, von Koch L, Conradsson D. Participation in social/lifestyle activities in people with multiple sclerosis: Changes across 10 years and predictors of sustained participation. Mult Scler J. 2019;1–10.
124. Sandoval AEG. Exercise in multiple sclerosis. Phys Med Rehabil Clin N Am [Internet]. 2013;24(4):605–18. Available from: http://dx.doi.org/10.1016/j.pmr.2013.06.010
125. Kraft GH. Rehabilitation still the only way to improve function in multiple sclerosis. Lancet. 1999;354(9195):2016–7.
126. Rietberg MB, Brooks D, Uitdehaag BMJ, Kwakkel G. Exercise therapy for multiple sclerosis. Cochrane Database Syst Rev. 2009;(4).
127. Latimer-Cheung AE, Martin Ginis KA, Hicks AL, Motl RW, Pilutti LA, Duggan M, et al. Development of evidence-informed physical activity guidelines for adults with multiple sclerosis. Arch Phys Med Rehabil [Internet]. 2013;94(9):1829-1836.e7. Available from: http://dx.doi.org/10.1016/j.apmr.2013.05.015
128. Snook EM, Motl RW. Effect of exercise training on walking mobility in multiple sclerosis: a meta-analysis. Neurorehabil Neural Repair [Internet]. 2009 Feb;23(2):108–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18948413
129. Romberg A, Virtanen A, Ruutiainen J, Aunola S, Karppi SL, Vaara M, et al. Effects of a 6-month exercise program on patients with multiple sclerosis: A randomized study. Neurology. 2004;63(11):2034–8.
130. Pilutti LA, Dlugonski D, Sandroff BM, Klaren R, Motl RW. Randomized controlled trial of a behavioral intervention targeting symptoms and physical activity in multiple sclerosis. Mult Scler J. 2014;20(5):594–601.
131. Oken BS, Kishiyama S, Zajdel D, Bourdette D, Carlsen J, Haas M, et al. Randomized controlled trial of yoga and exercise in multiple sclerosis. Neurology. 2004;62(11):2058–64.
132. Ewanchuk BW, Gharagozloo M, Peelen E, Pilutti LA. Exploring the role of physical activity and exercise for managing vascular comorbidities in people with multiple sclerosis: A scoping review. Mult Scler Relat Disord [Internet]. 2018;26(August):19–32. Available from: https://doi.org/10.1016/j.msard.2018.08.022
133. Negaresh R, Motl RW, Mokhtarzade M, Dalgas U, Patel D, Shamsi MM, et al. Effects of exercise training on cytokines and adipokines in multiple Sclerosis: A systematic review. Mult Scler Relat Disord. 2018;24(March):91–100.
134. Dalgas U, Stenager E. Exercise and disease progression in multiple sclerosis: Can exercise slow down the progression of multiple sclerosis? Ther Adv Neurol Disord. 2012;5(2):81–95.
135. Dorans KS, Massa J, Chitnis T, Ascherio A, Munger KL. Physical activity and the

incidence of multiple sclerosis. Neurology. 2016;87(17):1770–6.
136. Ghadirian P, Dadgostar B, Azani R, Maisonneuve P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis. Can J Public Heal. 2001;92(4):281–5.
137. Grover SA, Aubert-Broche B, Fetco D, Collins DL, Arnold DL, Finlayson M, et al. Lower physical activity is associated with higher disease burden in pediatric multiple sclerosis. Neurology [Internet]. 2015 Nov 10;85(19):1663–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26268901
138. Pilutti LA, Platta ME, Motl RW, Latimer-Cheung AE. The safety of exercise training in multiple sclerosis: A systematic review. J Neurol Sci [Internet]. 2014;343(1–2):3–7. Available from: http://dx.doi.org/10.1016/j.jns.2014.05.016
139. Tallner A, Waschbisch A, Wenny I, Schwab S, Hentschke C, Pfeifer K, et al. Multiple sclerosis relapses are not associated with exercise. Mult Scler J. 2012;18(2):232–5.
140. Motl RW, Dlugonski D, Pilutti L, Sandroff B, McAuley E. Premorbid physical activity predicts disability progression in relapsing-remitting multiple sclerosis. J Neurol Sci [Internet]. 2012;323(1–2):123–7. Available from: http://dx.doi.org/10.1016/j.jns.2012.08.033
141. Prakash RS, Snook EM, Motl RW, Kramer AF. Aerobic fitness is associated with gray matter volume and white matter integrity in multiple sclerosis. Brain Res [Internet]. 2010;1341:41–51. Available from: http://dx.doi.org/10.1016/j.brainres.2009.06.063
142. Kjølhede T, Siemonsen S, Wenzel D, Stellmann JP, Ringgaard S, Pedersen BG, et al. Can resistance training impact MRI outcomes in relapsing-remitting multiple sclerosis? Mult Scler J. 2018;24(10):1356–65.
143. Dalgas U, Langeskov-christensen M, Stenager E, Riemenschneider M, Hvid LG. Exercise as Medicine in Multiple Sclerosis — Time for a Paradigm Shift : Preventive , Symptomatic , and Disease-Modifying Aspects and Perspectives. Curr Neurol Neurosci Rep. 2019;9(88).
144. Waschbisch A, Wenny I, Tallner A, Schwab S, Pfeifer K, Mäurer M. Physical activity in multiple sclerosis: A comparative study of vitamin D, brain-derived neurotrophic factor and regulatory T cell populations. Eur Neurol. 2012;68(2):122–8.
145. Kornek B, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, et al. Multiple sclerosis and chronic autoimmune encephalomyelitis: A comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. Am J Pathol [Internet]. 2000;157(1):267–76. Available from: http://dx.doi.org/10.1016/S0002-9440(10)64537-3
146. Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH, et al. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol [Internet]. 2008 Sep;7(9):796–804. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18703004
147. Le Page C, Ferry A, Rieu M. Effect of muscular exercise on chronic relapsing experimental autoimmune encephalomyelitis. J Appl Physiol. 1994;77(5):2341–7.
148. Fainstein N, Tyk R, Touloumi O, Lagoudaki R, Goldberg Y, Agranyoni O, et al. Exercise intensity-dependent immunomodulatory effects on encephalomyelitis. Ann Clin Transl Neurol. 2019;6(9):1647–58.
149. Souza PS, Gonçalves ED, Pedroso GS, Farias HR, Junqueira SC, Marcon R, et al. Physical Exercise Attenuates Experimental Autoimmune Encephalomyelitis by Inhibiting Peripheral Immune Response and Blood-Brain Barrier Disruption. Mol Neurobiol. 2017;54:4723–37.
150. Le Page C, Bourdoulous S, Béraud E, Couraud PO, Rieu M, Ferry A. Effect of physical exercise on adoptive experimental auto-immune encephalomyelitis in rats.

Eur J Appl Physiol Occup Physiol [Internet]. 1996;73(1–2):130–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8861681
151. Klaren RE, Stasula U, Steelman AJ, Hernandez J, Pence BD, Woods JA, et al. Effects of exercise in a relapsing-remitting model of experimental autoimmune encephalomyelitis. J Neurosci Res. 2016;94(10):907–14.
152. Sharief MK, Hentges R. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N Engl J Med [Internet]. 1991 Aug 15;325(7):467–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1852181
153. Ruddle NH, Bergman CM, McGrath KM, Lingenheld EG, Grunnet ML, Padula SJ, et al. An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J Exp Med [Internet]. 1990 Oct 1;172(4):1193–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2212948
154. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. Neurology [Internet]. 1999 Aug 11;53(3):457–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10449104
155. Khademi M, Kockum I, Andersson ML, Iacobaeus E, Brundin L, Sellebjerg F, et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler [Internet]. 2011 Mar;17(3):335–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21135023
156. Maimone D, Gregory S, Arnason BG, Reder AT. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. J Neuroimmunol [Internet]. 1991 Apr;32(1):67–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2002092
157. Burman J, Svensson E, Fransson M, Loskog ASI, Zetterberg H, Raininko R, et al. The cerebrospinal fluid cytokine signature of multiple sclerosis: a homogenous response that does not conform to the Th1/Th2/Th17 convention. J Neuroimmunol [Internet]. 2014 Dec 15;277(1–2):153–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25457841
158. Kierkegaard M, Lundberg IE, Olsson T, Johansson S, Ygberg S, Opava C, et al. 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. J Neurol Sci. 2016;362:251–7.
159. Bansi J, Bloch W, Gamper U, Kesselring J. Training in MS: Influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. Mult Scler J. 2013;19(5):613–21.
160. Castellano V, Patel DI, White LJ. Cytokine responses to acute and chronic exercise in multiple sclerosis. J Appl Physiol. 2008;104(6):1697–702.
161. White LJ, Castellano V, McCoy SC. Cytokine responses to resistance training in people with multiple sclerosis. J Sports Sci. 2006;24(8):911–4.
162. White LJ, Castellano V. Exercise and brain health - Implications for multiple sclerosis: Part 1 - Neuronal growth factors. Sport Med. 2008;38(2):91–100.
163. Valdo P, Stegagno C, Mazzucco S, Zuliani E, Zanusso G, Moretto G, et al. Enhanced expression of NGF receptors in multiple sclerosis lesions. J Neuropathol Exp Neurol [Internet]. 2002 Jan;61(1):91–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11829348
164. Gold SM, Schulz KH, Hartmann S, Mladek M, Lang UE, Hellweg R, et al. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. J Neuroimmunol. 2003;138(1–2):99–105.
165. Zimmer P, Bloch W, Schenk A, Oberste M, Riedel S, Kool J, et al. High-intensity interval exercise improves cognitive performance and reduces matrix

metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial. Mult Scler J. 2018;24(12):1635–44.
166. Bernardes D, De Oliveira ALR. Regular exercise modifies histopathological outcomes of pharmacological treatment in experimental autoimmune encephalomyelitis. Front Neurol. 2018;9(NOV).
167. Radak Z, Suzuki K, Higuchi M, Balogh L, Boldogh I, Koltai E. Physical exercise, reactive oxygen species and neuroprotection. Free Radic Biol Med [Internet]. 2016;98:187–96. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2016.01.024
168. Smeyne M, Sladen P, Jiao Y, Dragatsis I, Jay Smeyne R, Author C, et al. HIF1α is Necessary for Exercise-Induced Neuroprotection while HIF2α is Needed for Dopaminergic Neuron Survival in the Substantia Nigra pars compacta HHS Public Access. Neuroscience. 2015;295:23–38.
169. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. Lancet [Internet]. 2012 [cited 2020 Jul 14];380(9838):219–29. Available from: http://dx.doi.org/10.1016/
170. Ekelund U, Tarp J, Steene-Johannessen J, Hansen BH, Jefferis B, Fagerland MW, et al. Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: Systematic review and harmonised meta-analysis. BMJ [Internet]. 2019 [cited 2020 Jul 14];366. Available from: http://dx.doi.org/10.1136/bmj.l4570
171. Croft AM, Palmer J V. Exercise and life expectancy. Lancet [Internet]. 2012;379(9818):800. Available from: http://dx.doi.org/10.1016/S0140-6736(12)60341-9
172. Nieman DC, Wentz LM. The compelling link between physical activity and the body ' s defense system. J Sport Heal Sci. 2019;8(3):201–17.
173. Suzuki K. Cytokine response to exercise and its modulation. Antioxidants. 2018;7(1).
174. Charlotte M, Severinsen K, Pedersen BK. Muscle–Organ Crosstalk: The Emerging Roles of Myokines. Endocr Rev. 2020;41(4):1–16.
175. Urenjak J, Obrenovitch TP. Neuroprotective potency of kynurenic acid against excitotoxicity. Neuroreport [Internet]. 2000 Apr 27;11(6):1341–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10817618
176. Agudelo LZ, Femenía T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, et al. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell. 2014;159(1):33–45.
177. Schlittler M, Goiny M, Agudelo LZ, Venckunas T, Brazaitis M, Skurvydas A, et al. Endurance exercise increases skeletal muscle kynurenine aminotransferases and plasma kynurenic acid in humans. Am J Physiol - Cell Physiol. 2016;310(10):C836–40.
178. Hillert J, Stawiarz L. The Swedish MS registry - clinical support tool and scientific resource. Acta Neurol Scand. 2015;132(S199):11–9.
179. Alping P, Piehl F, Langer-Gould A, Frisell T. Validation of the Swedish Multiple Sclerosis Register. Epidemiology. 2019 Mar;30(2):230–3.
180. Haukoos JS, Lewis RJ. The Propensity Score Use of the Method Why Were Propensity Methods Used? HHS Public Access. JAMA. 2015;314(15):1637–8.
181. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One. 2014;9(4).
182. Solier C, Langen H. Antibody-based proteomics and biomarker research — Current status and limitations. Proteomics. 2014;14(6):774–83.
183. Boven LA, Montagne L, Nottet HS, De Groot CJ. Macrophage inflammatory protein-

1alpha (MIP-1alpha), MIP-1beta, and RANTES mRNA semiquantification and protein expression in active demyelinating multiple sclerosis (MS) lesions. Clin Exp Immunol. 2000;122(2):257–63.
184. Ariel A, Lider O, Brill A, Cahalon L, Savion N, Varon D. Induction of interactions between CD44 and hyaluronic acid by a short exposure of human T cells to diverse pro-inflammatory mediators. Immunology. 2000;100:345–51.
185. Maurer M, Stebut E Von. Macrophage inflammatory protein-1. Int J Biochem Cell Biol. 2004;36:1882–6.
186. Hofer LS, Mariotto S, Wurth S, Ferrari S, Mancinelli CR, Delogu R, et al. Distinct serum and cerebrospinal fluid cytokine and chemokine profiles in autoantibody-associated demyelinating diseases. Mult Scler J - Exp Transl Clin. 2019;5(2).
187. Tejera-alhambra M, Casrouge A, Andrés C De, Seyfferth A. Plasma Biomarkers Discriminate Clinical Forms of Multiple Sclerosis. PLoS One. 2015;1–21.
188. Quandt J, Dorovini-Zis K. The Beta Chemokines CCL4 and CCL5 Enhance Adhesion of Specific CD4+ T Cell Subsets to Human Brain Endothelial Cells. J Neuropathol Exp Neurol. 2004;63(4):350–62.
189. Martínez Gómez J, Croxford JL, Yeo KP, Angeli V, Schwarz H, Gasser S. Development of Experimental Autoimmune Encephalomyelitis Critically Depends on CD137 Ligand Signaling. J Neurosci. 2012;32(50):18246–52.
190. Liu G, Gomes AC, Putheti P, Karrenbauer V, Kostulas K, Press R, et al. Increased Soluble 4-1BB Ligand (4-1BBL) Levels in Peripheral Blood of Patients with Multiple Sclerosis. 2006;412–9.
191. Barcellos-Hof MH, Dix TA. Redox-Mediated Activation of Latent Transforming Growth. Mol Endocrinol. 1996;10(9):1077–83.
192. Åivo J, Hänninen A, Ilonen J, Soilu-hänninen M. Vitamin D3 administration to MS patients leads to increased serum levels of latency activated peptide (LAP) of TGF-beta. J Neuroimmunol [Internet]. 2015;280:12–5. Available from: http://dx.doi.org/10.1016/j.jneuroim.2015.01.005
193. Hamaguchi M, Muramatsu R, Fujimura H. Circulating transforming growth factor- b 1 facilitates remyelination in the adult central nervous system. Elife. 2019;1–20.
194. Lee PW, Yang Y, Racke MK, Lovett-racke AE. Analysis of TGF-b1 and TGF-b3 as regulators of encephalitogenic Th17 cells: Implications for multiple sclerosis. Brain Behav Immun [Internet]. 2015;46:44–9. Available from: http://dx.doi.org/10.1016/j.bbi.2014.12.007
195. Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R. FGF21 induces PGC-1a and regulates carbohydrate and fatty acid metabolism during the adaptive. Proc Natl Acad Sci U S A. 2009;106(26):1853–8.
196. Pedersen BK. Physical activity and muscle–brain crosstalk. Nat Rev Endocrinol [Internet]. 2019;15(7):383–92. Available from: http://dx.doi.org/10.1038/s41574-019-0174-x
197. Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring H-U. Fibroblast Growth Factor 21 — Metabolic Role in Mice and Men. Endocr Rev. 2017;38(5):468–88.
198. Gómez-Sámano MÁ, Grajales-gómez M, Zuarth-vázquez JM, Navarro-flores F, Martínez-saavedra M, Juárez-león ÓA, et al. Fibroblast growth factor 21 and its novel association with oxidative stress. Redox Biol [Internet]. 2017;11(December 2016):335–41. Available from: http://dx.doi.org/10.1016/j.redox.2016.12.024
199. Furusawa Y, Uruno A, Yagishita Y, Higashi C, Yamamoto M. Nrf2 induces fibroblast growth factor 21 in diabetic mice. Genes to cells. 2014;864–78.
200. Hung H, Weihong P, Kastin AJ. The fasting polypeptide FGF21 can enter brain from blood. Peptides. 2007;28(12):2382–6.
201. Kuroda M, Muramatsu R, Maedera N, Koyama Y, Hamaguchi M, Fujimura H, et al. Peripherally derived FGF21 promotes remyelination in the central nervous system. J

Clin Invest. 2017;127(9).
202. Carlström K, Zhu K, Ewing E, Krabbendam I, Harris R, Falcão AM, et al. Gsta4 controls apoptosis of differentiating adult oligodendrocytes during homeostasis and remyelination via the mitochondria-associated Fas/Casp8/Bid-axis. BioRxiv. 2020;
203. Carlström KE, Chinthakindi PK, Espinosa B, Al Nimer F, Arnér ESJ, Arvidsson PI, et al. Characterization of More Selective Central Nervous System Nrf2-Activating Novel Vinyl Sulfoximine Compounds Compared to Dimethyl Fumarate. Neurotherapeutics. 2020;