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Safety and tolerability of fasting-mimicking diet in relapsing remitting multiple sclerosis.

Sicurezza e tollerabilità della dieta mima digiuno nella sclerosi multipla recidivante remittente.

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Summary

1 Multiple Sclerosis

1.1 Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS). MS is a heterogeneous, multifactorial, immune-mediated disease that is caused by complex gene–environment interactions.[1]

It is punctuated by fully or partially reversible episodes of neurologic disability, usually lasting days or weeks. Typical syndromes at presentation include monocular visual loss due to optic neuritis, limb weakness or sensory loss due to transverse myelitis, double vision due to brain-stem dysfunction, or ataxia due to a cerebellar lesion.[2]

MS primarily affects individuals in their early adult life, with an onset between 20 years and 40 years of age. It has a higher prevalence in women and significantly impacts their functionally, finances, and quality of life.[1,3]

In most patients, clinical manifestations and course of MS are reversible episodes of neurological deficits (known as relapses) that usually last for days or weeks characterize the initial phases of the disease. Over time, the development of permanent neurological deficits and the progression of clinical disability become more prominent.[1]

There is an improved understanding of the genetic, environmental, and lifestyle factors that contribute to the development of the disease. Both the innate and adaptive immune systems, with their effector cells, are known to influence the pathogenesis of MS.[3]

The exact etiology of MS is not known, even if the most accredited hypotheses suggest an autoimmune genesis, secondary to a response against a trigger event, which determines the “non-self” recognition of myelin antigens by autoreactive T lymphocytes.

Individual susceptibility is a factor of great importance and it is linked to the environmental and genetic backgrounds of the patient.

1.2 Epidemiology

Approximately three-quarters of people with MS are women, as is common in autoimmune diseases and suggests a possible role of environmental risk factors that mainly affect women.[1,2]

MS typically has an onset between 20 years and 35 years of age, although up to 10% of patients experience their initial demyelinating event during childhood or adolescence.[1]

The global distribution of MS generally increases with greater distance from the equator. In addition, while the disease is common in regions populated by people from northern Europe, this effect is modified according to where these individuals live in early life. Migration studies since the 1970s indicate that migration from low-risk to high-risk regions in childhood is associated with a low risk of developing MS.

Major environmental risk factors include geographic latitude, in particular low vitamin D levels and obesity during adolescence. Tobacco exposure, through active or passive smoking, as well as mononucleosis, are also associated with an enhanced risk of MS. Other, less-established risk factors include night work, excessive alcohol or caffeine consumption and history of infectious mononucleosis. Mononucleosis results from infection with Epstein–Barr virus in the postpubertal population, and MS eventually develops in only a minority of people with a history of mononucleosis. While other viruses have been suggested as potential causes of MS or MS–related disease activity, none have been definitively proven. Some of these viruses may act as molecular mimics, whereas others may interfere with mechanisms that normally limit self-reactive cells. [1,2]

The prevalence of familiar background is observed in people with an affected first-degree relative that have a 2 to 4% risk of MS, and concordance in monozygotic twins is 30 to 50%. Genomewide association studies, based on samples assembled from thousands of patients with MS and matched controls, have identified more than

200 gene variants that raise the risk of the disease, of which the most significant remains the HLA DRB1 and polymorphisms in IL2 and IL7R.[2]

The HLA region of chromosome 6 has been implicated in the development of hundreds of human diseases, including most autoimmune diseases.[3]

1.3 Pathogenesis

Although the etiology of MS remains unknown, the most accepted hypothesis by the scientific community regarding its pathogenetic process implies the existence of environmental triggers that, acting on a predisposing genetic substrate, activate an autoimmune response to CNS myelin damage.[1,3]

Among these environmental triggers, the following have been hypothesized:

- Vitamin D deficiency, especially during intrauterine life;
- Exposure to viral agents, such as measles virus, EBV, Herpes Virus (including HSV6) and some retroviruses, including HTLV1;
- Exposure to Chlamidia Pneumoniae bacteria.

Additionally, the association between an increased risk of contracting MS and an improvement in socio-economic conditions has been studied. This would result in a lack of exposure to certain pathogens in the first years of life, which could compromise the immune system that would develop self-aggression.

The autoimmune mechanisms thought to be involved in the pathogenesis include:

- Primitive damage to the myelin sheath by macrophages, NK cells, self-reactive antibodies and complement;
- Destruction of myelin or oligodendrocytes by T lymphocytes (CD8+)[4];
- Susceptibility of oligodendrocytes to toxicity developed by cytokines at the inflammatory site[5].

Tissue damage in multiple sclerosis results from a complex and dynamic interplay between the immune system, glia and neurons. Although there is debate about whether the root cause of multiple sclerosis is intrinsic or extrinsic to the Central Nervous System, studies in animal models have disclosed a critical role for adaptive immunity.[2]

It is activated already in the early stages of the disease and, by producing cytotoxic factors, induces apoptosis of oligodendrocytes, thus speeding up the demyelination process that characterizes MS.[2,4]

The myelin sheath is affected, and the nerve fiber suffers the most damage, undergoing axonal degeneration.

This is because demyelinated fibers are more susceptible to toxic insults from inflammation products.

1.4 Pathological Anatomy

The pathological hallmark of all MS phenotypes is the presence of focal plaques, also known as lesions. These areas of demyelination are typically located around post-capillary venules and are characterized by the breakdown of the blood–brain barrier (BBB).[1]

Their presence can vary in number and size, with diameters ranging from millimeters to centimeters. Based on their color and consistency, we can distinguish older, greyish lesions with increased consistency from more recent, less consistent, pinkish-colored lesions. The peculiar location of the lesion load can be found at the lateral ventricles, the floor of the aqueduct of the Silvio and the IV ventricle. Also frequently affected are the corpus callosum, optic chiasm and optic nerves, brainstem and spinal cord, especially in its cervical tract, with prevalent involvement of the dorsal columns and anterior sulcus.[1,2,3]

Microscopically, the plaques are characterized by areas without myelin, affected by the presence of an inflammatory cellular infiltrate and a variable degree of loss of oligodendrocytes and reactive astrogliosis depending on the stage of disease evolution.

The most recent lesions are characterized by a perivascular inflammatory infiltrate of T and B lymphocytes, followed by the advent of macrophages attacking the myelin sheath with associated axonal sparing.[4]

There is a progressive disappearance of the oligodendrocytes and myelin from the center of the lesion, replaced by astrocytic proliferation that gives rise to a tangle of fibrillar scar processes.

The inflammatory infiltrate tends to decrease to a very modest extent in advanced stages.

1.5 Physiopathology

The hallmarks of MS pathology are axonal or neuronal loss, demyelination, and astrocytic gliosis. Among these neuropathological characteristics, axonal or neuronal loss is particularly relevant because it is the main underlying mechanism of permanent clinical disability. Axonal loss occurs acutely in new inflammatory lesions, but also more slowly over time in chronically demyelinated lesions.[3]

Myelin is formed by the concentric retraction of the plasma membrane of oligodendrocytes along the axon and has the important function of allowing the rapid progression of the nerve impulse from one node of Ranvier to the next in a manner referred to as “jumping”. In demyelinated fibers, this type of conduction is no longer possible and the impulse is transmitted more slowly, by continuous propagation.

This slowing down is evidenced by loss of function and one of the most typical symptoms of the clinical manifestations of MS: fatigue. The vulnerability of demyelinated axon tracts to environmental factors such as increased temperature or mechanical stimuli has been highlighted, which explains the variations in the clinical presentation that can occur at different times during the day.

During the acute phase, the presence of demyelinated areas and inflammatory infiltrates along the axons leads to axonal distress and

subsequent functional loss. Partial recovery of function may occur if inflammation regresses, surviving oligodendrocytes attempt remyelination, or alternative conduction pathways are utilized. This pathophysiological condition corresponds to the clinical phase of the disease characterized by relapses and remissions (relapsing-remitting).

As the disease progresses, the lesions tend to become chronic and evolve into a gliotic-cicatricial pattern. This stage is characterized by a reduced inflammatory component and prolonged axonal distress, leading to irreversible cellular damage. At this point, a chronic-progressive phase of the disease is observed, whith symptoms not regressing and the neurological deficit stabilizing.[1,3,4,5,6]

1.6 Clinical Presentation

The clinical presentation of MS is heterogeneous and depends on the location of demyelinating lesions within the Central Nervous System (CNS).[1]

There is a wide range of symptoms that affect the person and differ in age of onset, symptoms of presentation, frequency of relapses, disease course and progression and disability caused. However, as areas of demyelination tend to be distributed in preferential locations, some symptoms recur more than others and will be more common and typical of MS.

1.6.1 Onset Symptomatology:

Typically, around 85% of patients experience an initial attack, characterized by an unpredictable episode of neurological dysfunction caused by demyelinating lesions in various areas such as the optic nerve, spinal cord, brainstem or cerebellum or the cerebral hemispheres.[1]

Motor manifestations are the initial symptoms in 30–40% of patients and affect almost all patients during the course of the disease. Motor

symptoms are characterized by pyramidal signs (such as Babinski sign, increased reflexes and clonus), paresis and spasticity.[1]

This symptom may manifest insidiously as a sensation of weakness or numbness in the lower limbs following prolonged exertion such as a long walk, with a tendency to recover after a period of rest. In other cases, there may be a subacute presentation with paraparesis or hemiparesis. The lower limbs are the most affected, often asymmetrically, while the onset of upper limb involvement is less common.

Optic neuritis is the first neurological episode in approximately 25% of patients. The risk that optic neuritis may evolve into a clinically definite MS increases with age, reaching a 50% between 10 years and 15 years after clinical onset. Optic neuritis is characterized by a partial or total visual loss in one eye with a central scotoma (a blind spot in the visual field), dyschromatopsia (color vision deficiency) and orbital pain worsened by eye movement.

The decrease in visual acuity is often preceded by supraorbital or eyeball pain and is reported by the patient as "blurred vision" or the sensation of "looking through frosted glass".

Sensory symptoms are very frequent in up to 43% of patients with MS and are mainly caused by myelitis or brainstem syndromes. Sensory symptoms include paresthesia and dysesthesia. They are described by the patient as tingling, numbness, pins-and-needles feeling, tightness, coldness and bandaging sensations or swelling of the limbs or trunk and are an expression of the involvement of the posterior chordae of the spinal cord, the spinothalamic pathways or the entry regions of the posterior roots.

The evocation of Lhermitte’s sign is also characteristic, described as an electric shock-like sensation radiating down the spine or into the limbs with neck flexion. These initial symptoms may regress in most cases, only to reappear spontaneously or in association with other symptoms and signs of CNS involvement as the disease progresses.

1.6.2 Overt Disease:

When the first clinical CNS demyelinating event lasting ≥24 hours is consistent with MS but does not fulfil the MS diagnostic criteria (see Table 1) it can be defined “clinically isolated syndrome” (CIS). We can speak of overt MS as soon as a diagnosis has been made.

During the disease course of MS, further clinical episodes can occur; these episodes last for ≥24 hours and occur in the absence of fever, infection or clinical features of encephalopathy. Symptoms of a clinical attack typically show an acute or sub-acute onset, worsen over days or weeks, reach a peak severity within 2–3 weeks and remit to a variable degree, ranging from minimal resolution to complete recovery normally 2–4 weeks after reaching maximum deficit.[1]

The patient’s symptoms can be categorized into different clinical syndromes, including mixed or generalized presentation (50%), characterised by involvement of the optic nerves, brainstem, cerebellum and spinal cord, spinal presentation (30-40%), in which deficits of deep sensitivity and motility are prevalent, and cerebellar presentation (5%), present in up to 70% of patients with MS, which include impairment in ocular movements such as nystagmus (involuntary eye movement), oscillopsia (a visual phenomenon in which items in the visual field seem to move) and diplopia (double vision), ataxia and gait imbalance, dysmetria (poor coordination) and decomposition of complex movements, slurred speech and dysphagia (difficulty swallowing).

Fatigue can be associated with relapses and can persist after the attack has subsided, but it can also be a feature of daily life and can be present for years.[1]

It is important to distinguish it from the symptoms associated with depression, another key issue to be dealt with in the MS sufferer.

This secondary depression arises from the patient's awareness of being affected by a progressively disabling disease, leading to uncertainty about the future and the fear of dependence during life.

Other symptoms include cognitive impairment, fatigue and affective disturbance. Overall, 40–70% of patients with MS have cognitive impairment, which can start in the earliest phases of the disease.[1]

Cognitive deficits can predict conversion to clinically definite MS in patients with CIS, are more frequent and more-pronounced in chronic progressive MS, worsen over time and affect patients' daily life activities. Common cognitive symptoms often include difficulties with information processing speed, episodic memory, attention, efficiency of information processing and executive function.[1]

Sleep disorders are very frequent and present themselves with insomnia, sleep apnea and restless leg syndrome.

Sphincter disorders are also common and can present themselves in two different ways:

- imperative urination, often associated with incontinence;
- difficulty initiating urination, with incomplete emptying of the bladder. The residue may be the site of origin of urinary tract infections.

The dysfunction usually becomes permanent late in the disease course, affecting 34–99% of patients.

Finally, deficits in sexual life are also noteworthy, from a simple decrease in libido to impotence and men with MS often have erectile dysfunction and impotence.

1.7 Progression

Depending on the disease course, three MS clinical courses can be distinguished:

- Relapsing-Remitting (RR)
- Secondary Progressive (SP)
- Primary Progressive (PP)

Clinically isolated syndrome (CIS) was not included in the initial MS clinical descriptors. CIS is now recognized as the first clinical

presentation of a disease that shows characteristics of inflammatory demyelination that could be MS but has yet to fulfill criteria of dissemination in time (DIT). Use of the 2010 revisions to the McDonald MS diagnostic criteria allows some patients with a single clinical episode to be diagnosed with MS based on the single scan criterion for DIT and dissemination in space (DIS), reducing the number of patients who will be categorized as CIS.[6]

A more complicated situation is the *radiologically isolated syndrome (RIS)*, where incidental imaging findings suggest inflammatory demyelination in the absence of clinical signs or symptoms. RIS was not considered an MS subtype per se since clinical evidence of demyelinating disease (a current criterion for MS diagnosis) is lacking and MRI findings alone may be nonspecific. However, RIS may raise the suspicion of MS, depending on the morphology and location of detected MRI lesions. Changes on brain imaging that are highly suggestive of demyelinating pathology carry the greatest risk of future MS clinical symptoms. An RIS patient with no obvious clinical signs or symptoms suggestive of MS should be followed prospectively. Until more information is available from prospective RIS cohorts, RIS should not be considered a distinct MS phenotype.[6]

Relapsing-Remitting Multiple Sclerosis (RRMS) is the most frequent clinical course and represents 80% of MS cases. It predominantly affects the female sex and typically has onset in youth. It is characterized by the appearance of isolated acute attacks that develop over days or weeks, followed by a generally complete recovery in the following weeks or months. The frequency of relapses is variable, although they will generally be less than one per year and more frequent in the early years of the illness. Between one crisis and the next, the patient is neurologically stable.

Secondary Progressive Multiple Sclerosis (SPMS) represents a later stage than the previous. After a variable number of years, about two

thirds of RRMS patients evolve towards SPMS; the transition is usually gradual.

Primary Progressive Multiple Sclerosis (PPMS) represents 20% of MS cases and what distinguishes it from the RR form is the progression of disability without the presence of acute attacks.
PPMS should remain a separate clinical course because of the absence of exacerbations prior to clinical progression, but it likely does not have pathophysiologically distinct features from relapsing forms of MS that have entered a progressive course (SPMS). Moreover, it has a more homogeneous distribution between the two sexes, it starts at a later age (average age 40 years) and the disability worsens faster.

This classification was partly updated thanks to the study published by Lublin in 2014, with the addition of two parameters: disease activity and disease progression.

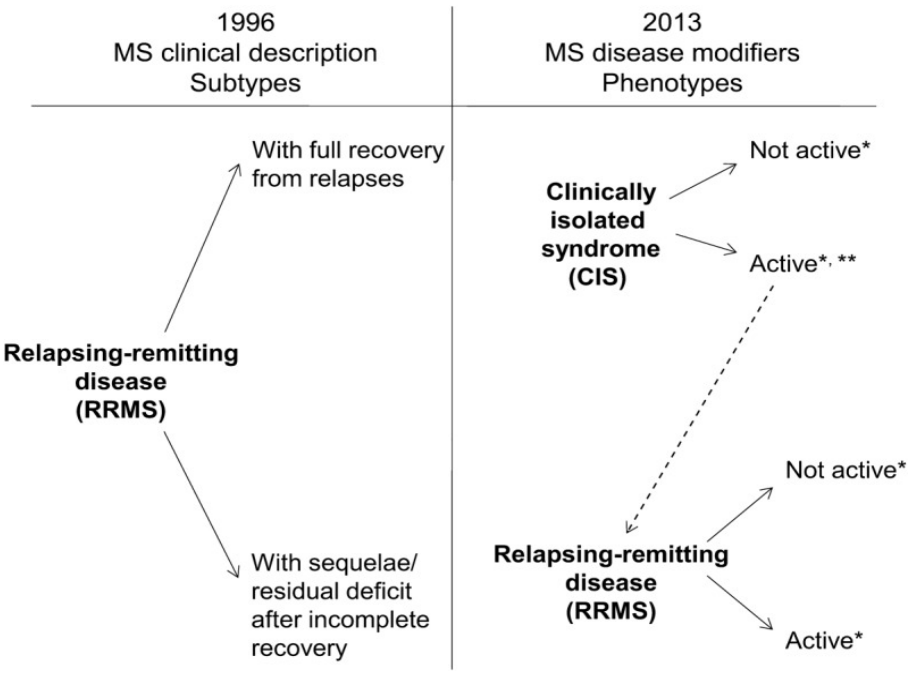


Fig.1: Change in the description of the relapsing/remitting MS phenotype.[6]

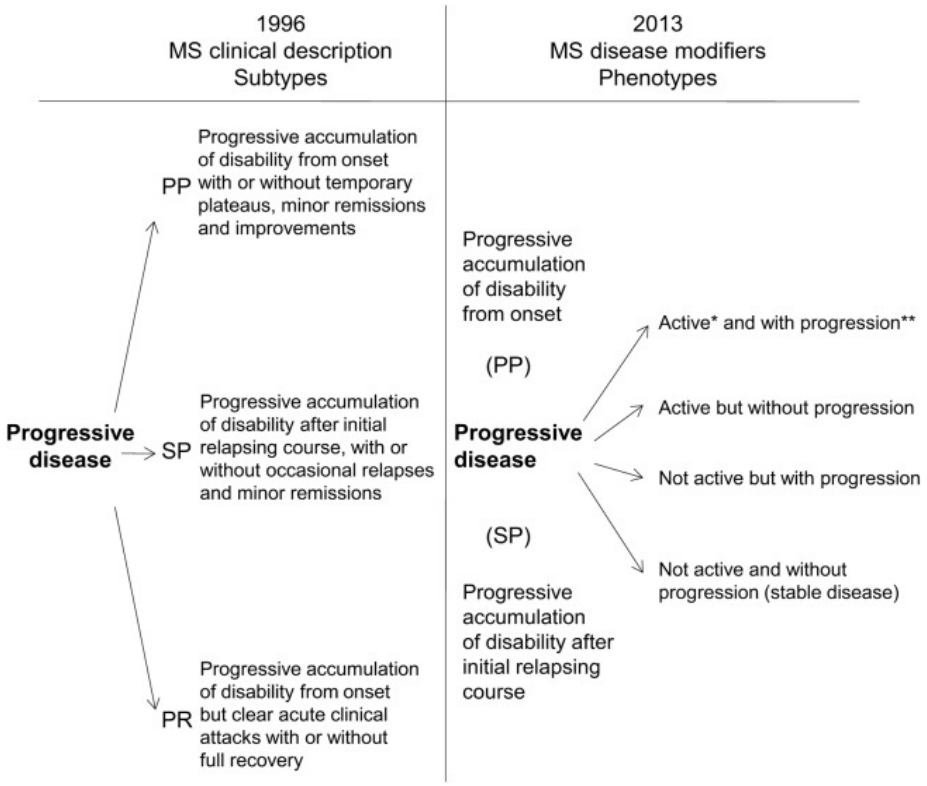


Fig.2: Change in the description of the progressive MS phenotype [6]

1.8 Paraclinical Investigations

Paraclinical and laboratory investigations are very important for diagnosis and for follow-up for MS patient.

1.8.1 MRI of the brain and spinal cord:

The most important diagnostic and prognostic MS biomarker, particularly early in the disease course, is MRI, which is currently the only technique that can interrogate the entire CNS in vivo.
By MRI, Inflammatory demyelination is easily visible, as are blood brain barrier changes that accompany its early development.[2]
The ability of this technique to identify clinically asymptomatic lesions makes it perfect for following the evolution of disease by documenting its dissemination in time and space.
Neurodegeneration in MS is best captured on MRI by measuring the size of the brain or spinal cord. An abnormally low "brain

parenchymal fraction", a measure of brain size relative to intracranial capacity, can be taken as surrogate evidence of prior disease-related brain atrophy.[2]

Lesions usually appear as multifocal, ovoid areas of increased signal on T2-weighted images, with lesions commonly located in periventricular, juxtacortical and infratentorial regions of the brain and in the spinal cord. The administration of gadolinium-based contrast agents and the acquisition of post-contrast T1-weighted images enable active lesions to be distinguished from inactive lesions; signal enhancement, which underlies active lesions, occurs owing to increased BBB permeability and corresponds to areas with ongoing inflammation. Lesions that persistently appear hypointense on post-contrast T1-weighted images, so-called black-holes, are associated with more severe tissue damage than that seen with lesions that do not appear dark on such images. This hypointensity is suggestive of demyelination and axonal loss.

In the diagnostic criteria for MS, MRI is used to confirm DIS or DIT for RRMS, and it has been included in the diagnostic criteria for PPMS.

The latest revision of the MS diagnostic criteria included the count of symptomatic lesions for the definition of DIS and DIT, which enables the simplification of the application of MRI criteria without losing their accuracy. The inclusion of cortical lesions as part of the diagnostic criteria is also relevant, as these lesions are specific for MS, although improvement in their detection is still necessary. At present, 18% of cortical lesions confirmed by pathological studies can be detected using MRI, most of which are type I lesions, whereas type III lesions (subpial) are difficult to detect even with advanced MRI techniques.

The growing application of MRI has substantially increased the identification of asymptomatic individuals with brain MRI abnormalities suggestive of MS, which is referred to as RIS (Radiologically Isolated Syndrome)

The probability that RIS patients will experience a first clinical

manifestation within five years is 34% and this risk increases if the subject in question is male, young and has spinal cord injuries.
More studies are necessary to further define RIS and the differential diagnosis of this disorder and to develop recommendations to monitor and eventually treat these patients.
Aside from use in diagnosis, MRI has also gained a fundamental role in monitoring treatment efficacy and in the early recognition of treatment-related adverse effects, for example, progressive multifocal leukoencephalopathy (PML) and other opportunistic infections.[1,2]

1.8.2 Examination of the Cerebrospinal Fluid:

is the fluid that surrounds, nourishes and protects the CNS. It is taken by lumbar puncture and is able to give us various information useful in diagnosing MS:

- presence of oligoclonal bands (BOC) of IgG, demonstrated in approximately 75-90% of MS patients. Their presence testifies to non-specific B immune activation by the nervous system and leads to an increase in the Link index, which is the product between the IgG quotient and the albumin quotient. BOCs are detectable using the isoelectrofocusil method on agarose gels. It is possible that the bands are absent at disease onset and may increase over time to varying degrees from patient to patient. It is essential, in order to exclude a peripheral origin, to simultaneously assess their presence on a serum sample.
- modest CSF pleocytosis up to a maximum of 50 cells/µl, present in about one third of cases, typically in the acute-onset forms. In most cases, however, the concentration of these cells, predominantly mononuclear, is normal (1-5 cells/µl).
- proteinorrachia generally mild.

If examination of the CSF reveals pleocytosis of more than 75 cells/µl or proteinuria of more than 1g/l, the MS hypothesis becomes less plausible.

1.8.3 Evoked Potentials:

allow the slowing of nerve conduction, caused by the loss of the myelin sheath, to be revealed, demonstrating the involvement of apparently unharmed structures. The most common tests used are:

- visual evoked potentials (VEPs), which are impaired in 90% of patients with a history of optic neuritis and in 70-80% of MS patients;
- the somatosensory evoked potentials (PES), which express nerve impulse conduction through the posterior chordae of the medulla and are impaired in 60% of sufferers;
- auditory evoked potentials (AEPs), which are now considered obsolete and no longer used.

At the level of these structures, the lesion is often subclinical, not clinically evident, so evoked potentials help to highlight it and further suggest the diagnosis of MS.

1.8.4 Blood tests:

Currently, no externally validated blood immune marker has adequate sensitivity and specificity to be used for MS diagnosis, probably reflecting the genetic and environmental heterogeneity of MS.

In any case they are useful to help the clinician in the differential diagnosis of MS.[1,2,3]

1.9 Diagnosis

The diagnosis of MS is based on the integration of clinical, imaging, and laboratory findings. Clinical expertise is necessary to demonstrate evidence of DIT and DIS and, importantly, to exclude other neurological conditions. MRI can provide this evidence and assist in excluding other conditions, allowing earlier diagnosis with increased certainty with successive versions of the diagnostic

criteria.[3]

The diagnostic criteria, known as the McDonald Criteria, which were further updated by the international community in 2017, make the diagnosis of MS quicker and simpler, ensuring high levels of sensitivity and specificity and enabling early treatment of the disease.[7]

The main changes compared to the 2010 criteria are:

- Oligoclonal bands in the CSF. BOC positivity in the CSF takes on the meaning of DIT.
- Lesions on MRI. Both asymptomatic and symptomatic lesions detectable on MRI are considered to determine dissemination in space or time. The only exception is MRI lesions of the optic nerve in a patient previously suffering from optic neuritis.[8]
- Location of lesions. Cortical lesions, which are evident on MRI, have been added to the criteria for determining the dissemination of lesions in space.[7,8]

McDONALD CRITERIA	
Number of lesions with objective clinical evidence	Additional data needed for a diagnosis of multiple sclerosis
≥2 clinical attacks: -Clinical evidence of more than 2 lesions. - Clinical evidence of one lesion, as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location.	None.
≥2 clinical attacks: -Clinical evidence of one lesion.	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MR: - New attack involving a new CNS region. - One or more SM-typical lesions in T2 sequences in at least two of the following areas of the CNS: (periventricular, cortical, iuxtacortical, infratentorial or spinal

	cord) regardless of whether these lesions are.
1 clinical attack: - Clinical evidence of more than 2 lesions.	Dissemination in time demonstrated by an additional clinical attack or by MRI or demonstration of CSF-specific oligoclonal band: - A second clinical attack. - Simultaneous presence on MRI of contrast-enhancing and non-contrast-enhancing lesions, symptomatic or asymptomatic. - Presence of a new T2 or contrast-enhancing lesion on a previous MRI, regardless of when the reference MRI was performed. - Positivity of oligoclonal bands (BOC) in the CSF.
1 clinical attack: - Clinical evidence of one lesion.	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI and dissemination in time demonstrated by an additional clinical attack or by an additional clinical attack or by MRI or demonstration of CSF- specific oligoclonal bands.
Insidious neurological progression suggestive of Primary Progressive Multiple Sclerosis Indicated by one year of disease progression (retrospective or prospective)	Two of the following three criteria are required: - One or more hyperintense lesions in T2 involving at least one of the following brain regions (periventricular, cortical, iuxtacortical, infratentorial). - Two or more hyperintense lesions in T2 involving the spinal cord, without distinction between symptomatic or asymptomatic lesions. - Positivity of oligoclonal bands (BOC) in the CSF.

Tab.1: The 2017 McDonald criteria for diagnosis of multiple sclerosis in patients with an attack at onset.[7]

1.10 Prognosis

Several biomarkers and prognostic factors associated with MS and with disability progression have been identified, including

environmental, genetic, clinical, laboratory and MRI features.[1]

Positive prognostic factors are female sex, optic neuritis or sensory symptoms at onset, total recovery after first attacks, less than 40 years at onset (excluding childhood onset), less than 2 relapses during the first year and minimal deterioration after 5 years of illness.

Negative prognostic factors are male sex, pyramidal or cerebellar symptoms at onset, action tremor, late onset and progressive disease course.

Pregnancy would also seem to reduce the number of relapses. This protective effect, however, ends with childbirth which, on the contrary, would lead to a "rebound effect", increasing the number of attacks in the following two months.

MRI of the brain provides important prognostic information especially in patients at the first demyelinating manifestation, a clinical presentation called CIS (clinical isolated syndrome):

RM presentation	Prognostic Significance
Presence of 3 or more typical lesions in T2	MS risk in 10 years: 70-80%.
Normal presentation	MS risk in 10 years:20%
2 or more gadolinium-capturing lesions	Highly predictive of future MS
Occurrence of new lesions at T2	
Increased enhancement at least 3 months after onset	

Tab.2: Association between MRI picture and relative prognosis.[8]

Fifteen years after disease onset, only 20% of patients will have no functional limitations and, indeed, half will have developed SPSM with a need for walking assistance. This level of disability will be reached by 80% of patients at 25 years after disease onset. There is also a 20% chance of being affected by the so-called "benign" form of MS. These patients will never develop disabilities

and even after 15 years, their neurological examination will be normal, indicating a good prognosis.[1,3,6]

One method used to assess the degree of disability is the Expanded Disability Status Scale (EDSS), developed by the American neuroepidemiologist John Francis Kurtzke.[9]

EDSS is of fundamental importance for following the progress of the disease and for checking whether there has been a positive response to therapy. Its score can range from 0 (physiological neurological examination) to 10 (death caused by MS). Although the latter occurrence is rather rare, it must be considered that survival at 25 years from onset is 85%.

The causes of death can be identified in an acute MS attack or, more frequently, in a complication of the disease.

EDSS	SIGNIFICANCE
0	Grade 0 in functional system – Normal neurological exam.
1	Grade 1 in more than one functional system – No disability, minimal signs (Babinski) or hypopallesthesia.
2	Grade 2 in one or two functional system – Minimal disability, hyposthenia or unsteady gait, visual or sensory disturbances.
3	Grade 3 in one or two functional or grade 2 in more than three – Moderate disability, monoparesis, moderate ataxia or combination of minor dysfunctions, fully preserved walking ability.
4	Grade 4 in one functional system or grade 3 in more than three fu – Relative severe disability, fully ambulatory without aid, self-sufficient, up and about some 12 hours a day.
5	Grade 5 in one functional system or minor grades combinations– Severe disability to impair full daily activities, ambulatory without aid or rest for about 200 meters.
6	Grade 3 or worse in combination in several functional systems - Intermittent or unilateral constant assistance (walking stick, crutch, or brace) required to walk about 100 meters with or without resting.

7	Grade 4 or worse in several functional systems rarely grade 5 only in the pyramid system -Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone.
8	Grade 4 or worse in most functional systems - Bedridden, but with effective use of arms.
9	Grade 4 or higher in most functional systems - totally impotent patients in bed.
10	Death due to MS.

Tab.3: Expanded Disability Status Scale (EDSS) [9].

1.11 Therapy

In the past 15 years, there has been a significant advancement in MS treatment. The effects of the disease that were long thought to be unavoidable, such as walking impairment that leads to dependence on a wheelchair, can now be delayed or, in rare cases with a particularly favorable prognosis, even prevented.

Reduction of inflammatory disease activity and its long-term clinical effects, control of disease relapse, and symptomatic treatment of typical signs like pain, exhaustion, and stiffness are the objectives of therapy.

It will be essential to determine which of the three possible modalities of MS (RR, PP, or SP) one is dealing with in order to begin treatment. This is due to the fact that each of these images represents a different disease-modifying treatment (DMT) therapeutic strategy.

There are now multiple DMTs approved to treat RRMS, but only one has been approved to treat progressive versions.

Numerous clinical trials, however, are in progress and may soon enable more therapy options and resources to address each of the potential patterns.

1.11.1 Relapsing-Remitting MS (RRMS) therapy:

To lower the risk of disease development in RRMS or CIS, it is essential to intervene as soon as feasible by utilizing the many DMTs that have been licensed for use in these types of MS. Interferon and Glatiramer-Acetate have been the first-line medications for the past twenty years for two main reasons: first, they have a very low risk profile (cases of severe adverse reactions to these medications are extremely rare), and second, they are less expensive than newer medications. Due to its manner of administration (subcutaneous injections) and associated adverse effects, however, their clinical efficacy was only moderate and patient tolerance was not high despite these positive characteristics. Due to its manner of administration (subcutaneous injections) and associated adverse effects, however, their clinical efficacy was only moderate and patient tolerance was not high despite these positive characteristics. The number of DMT choices that have been approved has increased, giving patients more options for treatment that will be increasingly individualized and take into account the efficacy, safety, and preferences of the patient. 'Escalation therapy' is the term for the therapeutic method that is currently most frequently utilized for RRMS. This involves starting treatment with a safe but only moderately effective medication, such as Interferon, Glatiramer-Acetate, Teriflunomide, or Dimethyl-Fumarate, and moving to another first-line medication in the case that the patient experiences unbearable adverse events. The plan is to switch to a more potent and efficient second-line medication in the case of fresh clinical relapses or MRI abnormalities. Finally, it is advised to proceed with an autologous transplant of hematopoietic cells in cases of severe illness if the patient does not react to standard DMT. Less than 1% of RRMS patients will actually get this procedure, thus it is quite uncommon. A different approach, known as "induction therapy," is predicated on the idea of starting patients on stronger, more effective medications, like alemtuzumab or ocrelizumab, to delay the onset of clinical impairment and irreversible CNS damage. By eliminating T, B, and myeloid cells, this method, utilized in patients with poor

prognosis variables upon diagnosis, enables a true reset of the immune system that is prone to self-reactivity. It is feasible to administer one or more of these cycles of induction therapy, which will be followed by a maintenance therapy regimen that is less strenuous. The type of DMT employed in this method must also be taken into consideration, as cycles of Natalizumab or Fingolimod that do not result in a full immunological reset must be followed by another extremely potent medication to reduce the chance of disease reactivation. Induction therapy's main drawback is the increased chance of serious adverse responses brought on by second-line medications, despite the fact that they are much more potent at treating the disease. While more recently approved DMTs are generally better tolerated by patients, they come with a much higher risk of developing severe adverse reactions, especially infectious ones. In contrast, older DMTs are characterized by a low risk of serious side effects but a higher frequency of less serious adverse reactions that compromise their tolerability. The most significant of these are HSV reactivations, respiratory and urinary tract infections, and particularly progressive multifocal leukoencephalopathy (PML). Patients receiving treatment with Natalizumab, Dimethyl-Fumarate, and Fingolimod were found to have the latter opportunistic brain infection brought on by the JC virus (present in 86% of the population in latent form). On the other hand, alemtuzumab raises the risk of developing autoimmune conditions, including Hashimoto's thyroiditis.

Finally, it is critical to evaluate the teratogenic risk connected to each DMT in female patients who intend to become pregnant. Glatiramer-acetate is the only DMT that is currently regarded as secure in this regard.[1,3,11,12,13]

1.11.2 Progressive MS (PMS) therapy:

DMTs used in RR formulations are unable to stop PMS from getting worse and progressing. The cytostatic medication Mitoxantrone was

given FDA approval for usage in the US in 2000, however its use was constrained because of its cardiotoxic and mutagenic effects. Natalizumab in PPMS and fingolimod in SPMS were subsequently studied, however the outcomes were unsatisfactory.[1,3]
However, a research on Ocrelizumab that demonstrated that the use of anti-CD20 drugs in PPMS resulted in a decreased risk of disability advancement as compared to placebo is what ultimately led to the current only approval of DMT against progressive forms.[12]
Ocrelizumab is the only DMT that has been approved to date against the PPMS form.

1.11.3 Relapse therapy:

The use of high dose corticosteroid medication is used to address disease relapses. This is due to the fact that such medications have shown promise in accelerating functional recovery and shielding the patient from the emergence of more serious abnormalities. The effectiveness of steroid medication over the long term is yet unknown, however this effect has been observed in the initial weeks after treatment. The administration of oral prednisone may be paired or not with intravenous methylprednisolone over a period of 3 to 5 days, according to current recommendations.
Plasmapheresis or intravenous immunoglobulin therapy may be used to treat relapses that do not respond to steroid therapy.

1.11.4 Symptomatic therapy:

Several medications are used to treat symptoms like pain, stiffness, walking impairment, sphincter abnormalities, and neuropsychiatric symptoms in MS patients in an effort to improve their quality of life. But there aren't many symptomatic treatments that have been proven to be truly successful. Among these are the anti-spasmodic cannabis Nabiximol and the walking-improving drug Dalfampridine. Among these are the anti-spasmodic cannabis Nabiximol and the walking-

improving drug Dalfampridine.

Chronic neuropathic pain is caused by damage to the sensory pathways, and SNRIs, tricyclic antidepressants, and gabapentin are recommended as first-line treatments. The second line is made up of opioids like tramadol or codeine. Oral antimuscarinic medications, either by itself or in combination with catheterization, are recommended for the treatment of symptoms associated to the last urinary tract. Botulinum toxin A bladder infiltrations and surgery may be utilized to treat more severe cases. Even though MS patients frequently have bouts of cognitive impairment, there is still a dearth of specialized treatment for these symptoms. It's feasible to stabilize cognitive function by combining specific DMTs (such Interferon, Natalizumab, and Fingolimod) with cognitive therapy. Additionally, some modest studies suggest that the combination of intracranial magnetic stimulation, cognitive behavioral therapy, alfacalcidol, a vitamin D analogue, and exercise may have clinical advantages in the fight against fatigue and psychiatric comorbidities.

In order to treat the wide range of disease-related symptoms, particularly the most severe ones that would restrict the patient in daily life, it is crucial to provide MS patients with therapy programs that are as complete as possible.[1]

2 Fasting-mimicking diet (FMD)

2.1 Introduction

Key determinants of aging and age-related illnesses include dietary composition and calorie intake.[13]

Metabolic syndrome is defined by co-occurrence of three of five of the following conditions:

- Abdominal obesity,
- Elevated fasting glucose,
- Elevated blood pressure,

- High serum triglycerides,
- Low levels of high-density lipoprotein (HDL) cholesterol,

and it is associated with a major increase in the risk of cardiovascular disease (CVD) and all-cause mortality.[14]

Despite the fact that prolonged fasting and very low calorie fasting-mimicking diets (FMDs) can reduce the incidence of diseases like cancer and multiple sclerosis in mice, randomized studies to test fasting's capacity to lower aging markers and major age-related diseases have not been conducted.[13,14] In organisms ranging from single-cell yeast to mammals, pro-growth signaling is reduced and cellular defense mechanisms are activated during prolonged fasting, which lasts for two days or longer and involves just water consumption.[14] This is done in mammals by, among other things, temporarily lowering blood glucose levels and levels of the hormone insulin-like growth factor 1 (IGF-1), which has been extensively researched for its effects on aging, cancer, and growth and development in addition to its role in metabolism and metabolism-related processes. A lower incidence of cancer, diabetes, and overall mortality in humans is linked to severe growth hormone receptor and IGF-1 deficits.[14]

Dietary restriction (DR) is an effective and reproducible intervention to increase healthy lifespan in various model organisms. The major DR regimens include caloric restriction (CR), intermittent fasting (IF), time-restricted feeding (TRF), restriction of specific macronutrients, ketogenic diets (KD), and periodic fasting (PF) or fasting-mimicking diets (FMDs) (see table 4).[21]

DR encourages metabolic and cellular changes that influence inflammation and oxidative stress, improve energy metabolism, and strengthen cellular defense. Fasting, the most extreme form of DR, which involves depriving oneself of all food but not water, can be used on a regular basis as cycles of extended fasting (PF) lasting two days or longer.[21]

A highly efficient method for protecting healthy cells and organs from various toxins and toxic situations while boosting the death of

many cancer cell types is PF cycles spanning two or more days but separated by at least a week of a normal diet.[21]

In mammals, this is achieved in part by temporarily reducing glucose and circulating insulin-like growth factor 1 (IGF-1), a hormone well studied for its role in metabolism, growth, and development, as well as for its association with aging and cancer. Severe growth hormone receptor and IGF-1 deficiencies are associated with a reduced risk of cancer, diabetes, and overall mortality in humans.[14]

Types		**Definition**	**Duration**
Calorie Restriction (CR)[15,16]		20–40% Reduction in total calorie intake.	Chronic
Dietary Restriction	Ketogenic Diet (KD)[17]	High-fat, low-carbohydrate diet. In human, diets with caloric contents of up to 75–80% fat and ≥ 15% protein are commonly used to treat various neurological disorders.	Chronic
	Fasting/Prolonged Fasting (PF)/Intermittent Fasting (IF)/Alternative Day (ADF)[18,19]	Fasting: Complete absence of food intake. PF refers to at least 2 and usually 3 or more consecutive fasting days, which can be repeated periodically. IF and ADF eliminate or greatly reduce daily intake of food/calories intermittently (usually every other day or every 2-3 days). Usually ADF refers to alternation of a day of either water only fasting or a	Periodic PF: 2 or more days/2-4 weeks IF/ADF: 1 day on 1 day off

		very low calorie diet.	
	Fasting mimicking diet (FMD)[20]	Formulations composition of macronutrients and micronutrients specifically formulated to trigger responses such as reduced glucose and insulin-like growth factor 1 (IGF-1) level, and increased ketone bodies, while maximizing caloric intake.	Periodic (2-7 days every 15-365 days)

Tab.4: Calorie and Dietary Restriction [21].

Numerous systems can be protected from aging and can live longer under various dietary restrictions. For instance, CR is typically used to describe a 20–40% reduction in caloric intake from ad libitum levels, whereas DR is a much more inclusive term that can be used to describe calorie or specific macronutrient restrictions.

IF, which is also synonymous with alternate day feeding (ADF), instead describes the alternation of a day of eating with a day of fasting on water only or eating extremely little calories. In contrast, periodic fasting (PF) is the practice of fasting for two or more days in a row on a regular basis. This might happen anywhere from once a week to many times per year.[21]

These definitions and classifications are particularly important in the identification and understanding of the dietary interventions that affect autoimmunity. In mice, PF or a 48–72 h FMD with low protein, sugar, and high fat can cause serum glucose levels to drop by at least 40%, and in humans, it can do so by at least 20%. These diets supply calories without interfering with the benefits of water-only fasting. Cells use liver glycogen as their primary energy source during the initial fasting period before switching to a metabolic mode

that uses non-hepatic glucose, ketone bodies made from fat, and free fatty acids. A significant decline in some growth factors is also brought on by PF, especially the insulin-like growth factor 1 (IGF-1), a crucial signaling molecule for cellular growth and an antagonist of both cell protection and regeneration.[21]

Fasting-based reductions in glucose as well as other nutrients and substances that cancer cells depend on improve the cancer cells' sensitivity to chemotherapy. As a result, poorly known partially overlapping mechanisms may cause PF to kill both cancer and immune cells, especially the more active autoimmune cells.[21]

2.2 Principles of fasting in MS

MS is an autoimmune disorder characterized by T cell-mediated demyelination and neurodegeneration of the central nervous system, whose exact etiology remains unclear but where environmental factors interact with genetic susceptibility.[21,23]

In experimental autoimmune encephalomyelitis (EAE), an animal model for MS, activated myelin-specific T_H1 and T_H17 cells cross the blood brain barrier and migrate into the CNS, where they are activated by local antigen presenting cells (APCs) and promote inflammation.[24]

During the activation phase, the antigen presenting cells migrate to the lymph nodes and present the immunodominant peptide to naive T cells, and the histocompatibility (MHC) Class II-restricted CD4+ T cells secrete many inflammatory cytokines such as IFN-y, TNFα, IL-6, and IL-17.[21]

During the effector phase, CD4+ T cells that recognize antigen proliferate, cross the blood-brain barrier and subsequently activate macrophages and microglia that cause demyelination, oligodendrocyte death, and axon degeneration, which eventually cause neurological damage.[21,24]

As the disease progresses, remyelination and regeneration of oligodendrocyte become inefficient and ultimately fail, resulting in

disease progression. Several MS treatment drugs have been effective in reducing immune responses, but their impact on long-term disease progression, accrual of irreversible neurological disability, and the function of the immune system remains largely unclear. The limitation of pharmacological immune-modulating treatments is due to both the relatively non-specific inhibition of immune responses leading to immunosuppression and the failure to repair the damaged myelin in the affected tissues.[21]

Although we are only beginning to understand the relationship between nutrients, fasting and autoimmune disorders, the dietary treatment of MS and other autoimmune diseases has high potential, since it may stimulate and take advantage of the ability of the organism to repair and replace its damaged cells without interfering with the function of normal cells and systems.[21]
Periodic cycles of prolonged fasting (PF) or of a fasting mimicking diet (FMD) lasting 2 or more days can increase protection of multiple systems against a variety of chemotherapy drugs in mice and possibly humans. Moreover, PF or FMD reverse the immunosuppression or immunosenescence of either chemotherapy or aging through hematopoietic stem cell-based regeneration. Chronic caloric restriction, a ketogenic diet (KD), and intermittent fasting have been shown to prevent EAE by reducing inflammation and enhance neuroprotection when administered prior to disease induction or signs but dietary interventions have not been reported as a therapy for EAE or MS or to promote myelin regeneration.[24]

2.3 Dietary restrictions in MS prevention in murine models

Virtually all chronic caloric restriction (CR) studies in mouse MS model have shown effects on prevention and not treatment, possibly because cycles of the combination of restriction and re-feeding as well as the severity, type and length of the restriction, not simply chronic CR optimizes the effects on MS pathology and symptoms.

Chronic moderate to severe CR (33–60%) has been shown to promote protective effects in the prevention of EAE in various MS models. After chronic CR, 33% or 40% of mice showed a minor decrease in the EAE induction and disease severity but were protected from EAE induced inflammation, demyelination, and axon injury. Interestingly, only severe CR (66% caloric restricted group) completely prevented EAE induction. Similarly, a chronic ketogenic diet, consisting of a 4:1 ratio of fat to carbohydrate and protein, has been reported to serve as a preventive measure against EAE. However, similar to the effect of chronic moderate CR, KD did not completely prevent the EAE disease induction but reduced the severity of the disease. Intermittent fasting (IF), where fasting is applied every other day, was also shown to help reduce EAE disease severity. Eight weeks of IF prior to the EAE immunization completely protected mice from EAE induction compared to 75% incident rate of *ad libitum* group. Although the exact mode of these protective effects is still under active investigation, these dietary interventions caused similar changes in immune response including a significant reduction in circulating and CNS-derived $CD4^+$and $CD8^+$ T cells and $CD11b^+CD45^+$ cells (macrophages and microglia) compared to the control diet group, and markedly reduced cytokines (IL-1β, IL-6, TNF-α, IL-12, IL-17) and chemokines (IFN-γ, MCP-1, MIP-1α, MIP-1β).[21]

2.4 Dietary restrictions in people with MS

It is crucial to find modifiable risk factors and therapies that may stop or slow disease development given the tremendous burden MS impairment causes. An increasing body of research shows a connection between poor diet, dysbiosis of the gut microbiota, and a number of autoimmune and inflammatory illnesses, including MS. Among people with MS, poor diet quality has been correlated with greater disability and symptom burden. Furthermore, obesity is a risk factor for developing MS in adults and children, is associated with a

greater degree of gray matter volume loss, and may be associated with worse clinical outcomes in people with established MS.[22]

The influence of diet on MS risk and prognosis may be multi-factorial, including potential changes to immune system functioning, cellular metabolic processes and/or gut microbiota composition.[22]

Interest in FMDs has recently grown with respect to the potential to impact MS. The term "fasting-mimicking diet" has been used to refer to a wide range of dietary therapies in which followers achieve a fasting state by engaging in extended periods of low or no calorie consumption. Among people with MS, calorie restriction may have clinical benefits, including improvement in fatigue and emotional wellbeing. Diet adherence is a major barrier to the feasibility of large, randomized-controlled diet trials; controlled feeding trials are costly and logistically complicated, and the results are harder to translate to the real world than self-directed dietary changes. Thus, it is interesting to explore clinical effects of dietary change in people with MS.[22]

In a human clinical trial, a 7-day cycle of a FMD followed by 6 months of a Mediterranean diet, was reported to be safe and feasible.[21] A chronic KD intervention also resulted in potentially positive effects on relapse-remitting MS patients.[21] Moreover, preliminary results suggest that both the FMD and the chronic KD were associated with positive changes in self-reported Health-Related Quality of Life. A very mild improvement in EDSS a 3 months follow-up in FMD (mean 0, range -1 – 0) and KD (mean 0, range -0.5 – 0) compared to what was reported by the control diet group (mean 0, range 0 – 0) has been reported.[21] Similar to the rodent study, the clinical trial also reports a reduction in lymphocyte upon FMD intervention which suggests that the diet may work in a similar manner in mice and humans.[21] However, whether or not FMD cycles will reduce MS pathology in humans is still unclear and must be tested in larger randomized clinical trials. One of the possible mechanisms of the FMD-dependent modulation of autoimmunity

involves the up-regulation of serum glucocorticoid, adiponectin and the reduction of IL-6 and leptin. RRMS patient had significantly higher leptin and resistin levels and lower levels of adiponectin and Treg cells. Therefore, PF and FMDs may stimulate endogenous production of glucocorticoid and adiponectin which may contribute to a system-wide suppression of specific immune responses.[21]

Three randomized-controlled diet studies, at Johns Hopkins MS Center between 2015 and 2017 were conducted to evaluate the safety and feasibility of FMDs in people with RRMS. The first involved altering the timing or amount of calories in MS.[22] Of 36 participants were originally enrolled in the first trial; 31 of the 36 participants completed the initial 8-week controlled feeding portion and continued into the self-directed ICR portion of the study. Participants were randomized to follow either a daily continuous calorie restriction (CCR) consisting of 78% of caloric requirements for 7 days per week, an intermittent calorie restriction (ICR) consisting of 25% of caloric requirements for 2 days per week and 100% for the remaining 5 days per week, or a control diet with 100% of caloric requirements for 7 days per week. The second consisted of choosing between two different types of diets, CCR or ICR. Twenty-six patients initially consented to the study, and of the 19 who completed a baseline visit, 11 selected CCR and 8 selected ICR.

Participants were then allowed to select their preferred diet, as adherence research in pharmacologic studies has demonstrated patient choice significantly increases likelihood of adherence.

The third one consisted in following TRF diet (N=12), to consume all calories in an 8-h interval, resulting in a 16-h fasting period daily, or a control diet (N=12), that consists in no change to diet. 22 participants completed the study, and no adverse events were reported.

The diets were well tolerated and the results provided evidence that CCR, ICR and TRF are safe among people with MS. These results showed how calorie restriction leads to weight loss and may improve

aspects of health-related quality of life in MS patient populations. They demonstrated improvements in several areas including fatigue or depressive symptoms associated with the dietary interventions. In addition, the inclusion of multiple variants of a diet that mimics fasting and different levels of clinical support for participants added a broader scope of data to the existing literature than just one diet variant. However, large, randomized controlled studies are still needed to provide strong evidence regarding whether dietary modification in people with MS is of clinical benefit.[22]

Another study showed the effects of intermittent or daily CR diets in people with MS on several relevant classes of potential biologic mediators that included adipokines, various T cell subsets and the circulating metabolome. Leptin and adiponectin did not change significantly in individuals randomized to either CR diet, despite several previous studies implicating a link between fasting and lowering of leptin levels and increasing of adiponectin, while randomization to an intermittent CR diet was associated with alterations in T cell subsets and in several classes of biologically relevant lipid metabolites.[23]

The observed changes in naïve T cells are consistent with EAE studies of intermittent CR, in which the relative number of naïve T cells increases with fasting in addition to a shift to T regulatory cells (Tregs) away from effector T cells, including a reduction in Th1 cells.[23]

Since these T cell populations can then produce other pro-inflammatory mediators that can impact overall T cell function, this offers a potential explanation for the immunological changes observed.[23]

2.5 Fasting mimicking diets as a treatment for MS

Some of the major limitations of the chronic dietary restrictions listed above are:

1) they would be very difficult to adopt for the great majority of the population,

2) they can cause both protective and detrimental effects including impaired immune responses and would healing,

3) they are effective in the prevention but not treatment of MS. Furthermore, these dietary interventions fail to address one of the core aspects of MS treatment, which is the need to stimulate both the regeneration of functional white blood cells and the remyelination at the demyelinated lesions either by stimulating myelin production or regenerating oligodendrocyte from oligodendrocyte precursor cells. Periodic treatment with a fasting mimicking diet (FMD) with a very low calorie and protein content has the potential to overcome the limitations listed above. FMD cycles were shown to attenuate EAE symptoms by modulating immune cells and promoting oligodendrocyte precursor cell regeneration. A study showed that a periodic fasting mimicking dietary intervention has potent MS treatment effects in mice but also that it has the potential to minimize side effects as well as compliance issues. FMD cycles not only resulted in a reduction in dendritic cells which are known to play an important role as antigen presenting cells (APC) that secrete cytokines responsible for activating T lymphocytes, but also reduced circulating MOG specific $CD4^+$ T cells, Th1 and Th17 cells and reduced serum cytokines such as IFN-γ, IL-17 and TNF-α. Furthermore, the FMD treatment increased anti-inflammatory $CD4^+$ Treg. It was previously shown that during the cycles of the prolonged fasting, the immune cells undergo system-wide apoptosis followed by hematopoietic stem cell based regeneration upon re-introduction of nutrients (re-feeding period) in a IGF-1-PKA dependent manner. Similarly, FMD cycles cause apoptosis of autoreactive T cells, which are replaced by newly generated naïve T cells during the re-feeding period. More importantly, the FMD treatments promoted oligodendrocyte precursor cell dependent regeneration, which is known to participate in remyelination of demyelinated axons.[21]

2.5.1 Impact of Diet on serum neurofilament light chain in MS:

Another study explored the impact diets on serum neurofilament light chain (sNfL) levels in patients with relapsing-remitting MS. In MS, effective therapeutic strategies and sensitive biomarkers to evaluate drug efficacy and disease course are urgently needed. Serum neurofilament light chain protein (sNfL) has recently been suggested as a promising candidate for a reliable, easy-to-use biomarker of neuroaxonal damage that accurately detects changes over both long and short time intervals in MS. After axonal injury, NfL is elevated in both the CSF and in the peripheral blood, where it can be measured by highly sensitive single molecule assays. The observed diet-induced improvement of neuronal resistance and axonal survival are clearly neuroprotective, but the underlying mechanisms remain elusive. In the study is investigated whether AKD and CR in comparison to common diet (CD) may affect neurodegeneration as measured by sNfL levels in MS.[24]

NfL levels have previously been demonstrated to decrease following the start of the therapy, serving as a measure of neuroaxonal integrity and a treatment response marker. In agreement with this, the findings suggest that AKD may affect sNfL levels in MS patients. To assess the long-term effects of repeated fasting cycles on sNfL levels in MS, more research is required. This suggests that AKD may benefit the course of disease in MS. However, more comprehensive studies with an expanded time span are needed to examine whether effects could relate to the diet itself rather than its impact on MS. Overall, the study suggests that an AKD offers an avenue to impact sNfL levels, which seems to be a promising biomarker in neuroinflammatory diseases, supporting the use of dietary interventions as a treatment tool for MS.[24]

2.6 Fasting mimicking diet as a treatment for other diseases

2.6.1 Diabetes:

Type 1 diabetes is an autoimmune condition that is frequently detected in children and young adults. It results in the loss of the insulin-producing pancreatic beta cell in the islets of Langerhans, which prevents the body from making insulin. Infiltration of innate and adaptive immune cells, which release cytokines that encourage beta cell death and increase the infiltration of islet-specific T cells, is linked to type I diabetes. Specifically, CD4+ or CD8+ infiltrating T lymphocytes are crucial in the development of type 1 diabetes mellitus. Studies employing both human and animal models of diabetes have shown that both CD4+ and CD8+ T cells and macrophages are necessary for the damaging autoimmune process in type 1 diabetes. Although DR has been extensively researched for the prevention and treatment of type 2 diabetes, nothing is known about how various DR forms affect autoimmune type 1 diabetes.[21]

2.6.1.1 Dietary restriction in diabetes type 1 murine models:

Studies have revealed that CR enhances glucose homeostasis and lowers oxidative stress and lipid peroxidation in the type 1 diabetic (T1D) rat model caused by streptozotocin (STZ). Prior to STZ-induced diabetes, chronic CR (30% less calories per day) for 9 weeks demonstrated protection against diabetic insults. In the plasma of streptozotocin-induced diabetic rats, CR suppresses the up-regulation of inflammatory cytokines (IL-1, IL-4, and IL-6) and TNF- and activates IL-10 and haptoglobin. IF, which was carried out for 30 days from 5 p.m. to 8 a.m., reduced the weight gain that is frequently seen in the pancreas, liver, and kidneys of STZ-treated rats. In the pancreas of STZ-induced diabetic rats, IF increased glucose tolerance, insulin sensitivity, and the percentage of apoptotic B cells. Additionally, it has been demonstrated that changing the

amount of protein in the diet can make diabetes worse. The non-obese diabetic (NOD) mice showed that a high-protein diet hastened the onset of illness in spontaneous autoimmune models. It has been demonstrated that the forms of dietary protein have a significant influence on the prevalence of diabetes in NOD mice. NOD mice fed meat or casein experienced an early onset of the illness, while mice fed casein hydrolysate, a denatured version, or a diet high in lactalbumin were comparatively protected.

The reprogramming of pancreatic islet cells caused by FMD cycles has recently been demonstrated to be able to restore insulin insufficiency in mice models of Type 1 and Type 2 diabetes by inducing gene expression patterns similar to those seen during fetal development. In human cells obtained from autoimmune type 1 diabetes patients, fasting mimicking circumstances also corrected insulin deficiency abnormalities, suggesting that FMD has the potential to treat human diabetes. Therefore, knowledge of the connection between nutrition and the T1D-related autoimmune is still in its infancy. FMD cycles, however, seem to have a lot of potential for treating both Type 1 and Type 2 diabetes. Although it is unknown if this impact involves pancreas regeneration, a recent pilot trial of patients who underwent three monthly cycles of the FMD revealed a sustained decrease in fasting glucose. These findings require confirmation from more animal and randomized research in order to be used as the basis for nutritional treatment of autoimmune diabetes.[21]

2.6.1.2 Intermittent fasting in diabetes type 2 rodent models:

In rat models of type 2 diabetes, IF can both prevent and treat the condition. It is possible to prevent the development of insulin resistance and diabetes in sand rats fed a high-fat diet by keeping them on a time-restricted feeding schedule of 8 hours per day (TRF diet, which involves 16 hours of fasting per day). The same is true for C57BL/6 mice, which develop hyperinsulinemia, obesity, and

systemic inflammation when fed a high-fat diet ad libitum. All of these conditions can be avoided by limiting food availability to 8 hours per day. Due to the fact that mice fed for just 8 hours per day consume the same quantity of food as control mice fed ad libitum, the latter anti-diabetic benefit of TRF is not the result of caloric restriction. Mice with decreased brain-derived neurotrophic factor (BDNF) levels are hyperphagic, develop insulin resistance, and develop diabetes, same like leptin-deficient mice and leptin receptor mutant mice. Leptin receptor mutant mice received daily intraperitoneal injections of BDNF, which cured diabetes and obesity. Glucose tolerance is normalized and circulating glucose, insulin, and leptin levels are lowered in diabetic BDNF+/ mice kept on an ADF diet. It's interesting to note that IF, through a mechanism involving the maintenance of pancreatic beta-cells, can improve the insulin deficit and glucose intolerance in a rat model of type I diabetes. Although not yet proven, it is likely that IF increases cellular stress resistance, as has been noted in investigations of IF's effects on other cell types, protecting-cells. Increased insulin receptor signaling sensitivity makes it easier for insulin to drive glucose uptake by muscle and liver cells, as well as perhaps other cell types including neurons. This is the physiological and molecular process through which IF prevents and cures diabetes. Other signaling pathways that may be altered by IF in one or more cell types include those that control mTOR signaling, mitochondrial function, mitochondrial biogenesis, CREB, BDNF, and autophagy pathways. Diabetes causes inflammation in a number of organ systems, and IF's ability to reduce inflammation may help explain why it has anti-diabetic properties.[27]

2.6.1.3 Intermittent fasting in type 2 diabetes in humans:

There are minimal data on the effects of IF versus continuous energy restriction (CER) on glucose homeostasis amongst overweight/obese individuals with type 2 diabetes.[27]

The first study reported that a four day IF led to comparable reductions in percentage body fat and reductions in HbA1c to an isocaloric CER. The second study assessed the effect of enhancing a standard 25% CER diet with periods of IF, (75% ER either 5 days/week every 5 weeks or 1 day/week for 15 weeks). Predictably, additional periods of ER increased weight loss. The 5 days/week every 5 weeks intervention resulted in the greatest normalization of HbA1c, independent of weight loss suggesting a potential specific insulin-sensitizing effect of this pattern of IF added to CER.[27]

The two studies of a 2 days/week IF mentioned previously have reported greater reductions in insulin resistance versus CER amongst overweight and obese non-diabetic subjects. In the first study the subjects on the IF diet exhibited a 25% greater reduction in insulin resistance compared to the CER group when measured on the morning after five normal feeding days, with a further 25% reduction in insulin resistance compared with CER on the morning after the two energy restricted days. These differences in insulin sensitivity occurred despite comparable reductions in body fat between the groups.[27]

Thus, IF has been reported to have variable effects on peripheral and hepatic insulin sensitivity which may be different in obese and normal weight subjects and may be gender-specific. Further studies are required using more robust measures of insulin sensitivity.[27]

2.6.2 Cardiovascular diseases:

IF has been shown to offer significant cardioprotective benefits in trials using rats and mice. In a model of myocardial infarction (MI; coronary artery ligation), rats kept on ADF for three months prior to MI showed a smaller infarct size and a 75% lower number of apoptotic cells in the vulnerable area (penumbra) than the ad libitum control rats. Rats on the ad libitum diet experienced left ventricular remodeling and infarct expansion, but not those on the ADF diet, according to post-MI longitudinal echocardiographic investigations.

Similar to rats, ADF shielded the mouse heart from harm brought on by Mi. ADF did not shield the hearts of mice with defective autophagy (Lamp2 heterozygous mutant mice), in contrast to mice of the wild type. Instead, ADF increased myocardial damage in Lamp2-deficient animals, proving that ADF's cardioprotective effects are mediated via autophagy stimulation. When started 2 weeks after a MI caused by blocking the left coronary artery, IF has also been shown to significantly increase survival and cardiac function recovery in rats. Less than 25% of the rats on the typical ad libitum diet survived the 8-week post-MI period, whereas more than 75% of the animals on the ADF diet did. Given that levels of HIF-1, BDNF, and VEGF were significantly higher in the cardiac tissue of rats on IF compared to those on the control diet, the latter study's data about the mechanism of action of IF is consistent with the participation of hormesis/adaptive cellular stress responses.

When ADF was started in 2-month-old rats, it shielded the heart from age-related increases in fibrosis, oxidative stress, and inflammation. ADF inhibited age-related elevations in ERK1/2 and PBK7 kinases in cardiac cells as well as changed STAT3 transcription factor activity. The advantages of IF on heart function as we age seem to be very conserved. On the other hand, it was shown that after 6 months of being kept on ADF, rats show decreased cardiac reserve and a decrease in left ventricular diastolic compliance. Because the rats on ADF weighed significantly less than the rats fed ad libitum and may consequently require less cardiac output to fulfill their needs when spending a sedentary life in laboratory cages, the interpretation of the latter data is questionable.[27]

Humans are more susceptible to cardiovascular disease and stroke if they have hypertension, low heart rate variability, insulin resistance, and hyperlipidemia. In laboratory animals, IF lowers blood pressure, raises heart rate variability, and lowers insulin resistance. The improved vasodilation that is dependent on vascular endothelial cells may contribute to the blood pressure lowering. The increased activity of brainstem cholinergic cardiovagal neurons may be the

cause of the increased heart rate variability in rats maintained on ADF. In animals fed ADF and TRF diets, circulation levels of cholesterol and triglycerides are decreased. TRF shields mice from metabolic syndrome and obesity brought on by atherogenic diets like high fat + glucose and high fructose diets. The later effects of TRF are connected to decreases in circulating leptin and triglyceride levels, hepatic triglyceride content, and proinflammatory cytokine levels in adipose tissue.[27]

2.6.2.1 Cardiovascular diseases in humans:

Several studies have been conducted to evaluate the effects of modified ADF on cardiovascular risk factors in overweight and obese subjects. In the first study, ADF for 2 months resulted in a reduction in resting heart rate and circulating levels of glucose, insulin and homocysteine, all factors favourable to cardiovascular disease risk. In the second, 2 months of ADF reduced fat mass, total cholesterol, LDL-cholesterol and triglyceride concentrations. However, few studies have evaluated the relative effects of IF and CER on cardiovascular risk markers. Randomised comparisons of IF and CER reported equivalent reductions in blood pressure and triglycerides and an increase in LDL particle size.[27]

2.6.3 Rheumatoid Arthritis:

Rheumatoid arthritis (RA) is a long-lasting, systemic inflammatory condition marked by synovial hyperplasia, the development of autoantibodies, the deterioration of bone and cartilage, as well as the deformity and degeneration of several joints. There are many complex genetic and environmental variables involved in the etiology of RA. In both the early stages and later stages of the disease, RA involves an abnormal pathway of T cell activation, similar to other autoimmune diseases. It is now understood that the pathogenesis of RA involves a more nuanced autoimmune response

and cannot be fully explained in terms of the traditional antigen-driven proliferation of effector T cells. It is widely acknowledged that CD4+ T effector cells (both Th-1, 2, and 17), which can be found in RA synovial joints, are directly related to RA. Additionally, RA patients have a premature immune aging phenotype that includes an accumulation of CD4+CD28 T cells, telomeric shortening in hematopoietic stem cells, defects in naive CD4 T cell proliferation, premature telomere loss in naive CD4 T cells, loss of telomerase in T cells, and impaired DNA damage repair due to ATM insufficiency. Various dietary therapies have been demonstrated to lessen and perhaps even reverse the symptoms of RA.[21]

2.6.3.1 Dietary restriction in rheumatoid arthritis patients:

Numerous clinical investigations have been carried out to determine the effectiveness of fasting as an alternate treatment for RA. Patients were divided at random into two groups: those who followed a control diet and those who fasted once for a period of between 7 and 10 days before switching to a vegan diet for 3.5 months. All clinical measures and half of the laboratory markers, like erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), which are associated to the severity of RA disease, significantly improved in the fasting group's patients' reports. In a related trial, patients were given one of two treatments: a strict vegan diet followed by a fasting period (7–10 days). The fasting group's patients reported a considerable reduction in discomfort and an improvement in symptoms. Therefore, either an FMD or periodic fasting may be used to treat RA, albeit bigger randomized studies are required to explore this idea. It will be crucial to assess the impact of FMD cycles that are administered every one to two months on RA patients, whether or not there is a requirement for them to make significant dietary modifications, which are unlikely to be practical for the majority of patients.[21]

2.6.4 Neurological disorders:

Advancing age is the major risk factor for Alzheimer's disease (AD), Parkinson's disease (PD) and stroke. The degeneration and death of neurons that occurs in each of these disorders is believed to involve impaired mitochondrial function, oxidative damage, impaired lysosome function and dys-regulation of cellular calcium homeostasis. In the 1990s, studies were initiated to test the general hypothesis that, because aging is the major risk factor for neurodegenerative disorders, and because IF can counteract aging processes, IF may protect neurons in animal models of the disorders.[27]

Alzheimer's disease (AD) is characterized by psychological issues as well as increasing memory loss. The hippocampus, entorhinal cortex, basal forebrain, frontal and parietal lobes, as well as other brain regions involved in cognition and emotion, are responsible for the malfunction and death of neurons that lead to the behavioral abnormalities in AD. The aberrant buildup of extracellular deposits of amyloid -peptide (A), a 40–42 amino acid proteolytic cleavage product of the amyloid precursor protein (APP), causes synapses and neurons in these brain areas to degenerate. By causing membrane-associated oxidative stress and upsetting cellular ion homeostasis, A may lead to synapse dysfunction and neuronal degeneration.[28]

In animal models of stroke, Parkinson's disease, and Huntington's disease, prior research has demonstrated that CR and IF diets are neuroprotective and enhance functional outcome. According to the animal research, CR and IF may help the brain by lowering oxidative stress levels and boosting cellular stress resistance systems. Reduced food intake may also protect against AD, according to data from studies of human populations and animal models. According to a different study, AD risk is increased by midlife obesity. Additionally, conditions like diabetes and cardiovascular disease, which are brought on by eating too many calories, are linked to a higher risk of AD. Recent research suggests that CR can inhibit a crucial

pathogenic step in AD by reducing the development of amyloid pathology in the hippocampus and cerebral cortex of transgenic mice overexpressing FAD APP mutations. However, it is unclear how CR and IF diets may affect how AD patients develop cognitive dysfunction.[28]

Numerous studies have shown that, when initiated prior to the ischemic insult, ADF can reduce brain damage and improve functional outcome in animal models of stroke. The cellular and molecular mechanisms by which IF protects brain cells against a stroke have not been fully established but involve up-regulation of expression of neurotrophic factors (BDNF and FGF2), antioxidant enzymes (heme oxygenase 1) and protein chaperones (HSP70 and GRP78). Reduced inflammation may also mediate the beneficial effects of IF in stroke models as indicated by reduced levels of proinflammatory cytokines (TNFα, IL1-β and IL6) and suppression of the "inflammasome". Indeed, IF can attenuate cerebral oxidative stress and cognitive impairment induced by lipopolysaccharide in an animal model of systemic inflammation. Reductions in levels of leptin and increased levels of ketones may also contribute to neuroprotection by IF in stroke models. It remains to be determined whether post-stroke IF will modify functional outcome/recovery in animal models, which will be critical to know when considering whether or not IF is likely to benefit human stroke patients.[27]

IF has been reported to improve outcome in animal models of traumatic injury to the nervous system, as well as in models of peripheral neuropathy. In rat models of incomplete cervical spinal cord injury and thoracic contusion injury, ADF initiated prior to the injury and continued thereafter significantly improved functional outcome and reduced spinal cord lesion size. ADF was also beneficial when initiated after thoracic contusion spinal cord injury. However, in a mouse model of spinal cord injury ADF initiated after the injury did not significantly affect functional outcome or spinal cord lesion size. The reason why ADF was effective in the rat models, but not in the mouse model, is unclear and merits further

investigation. As with spinal cord injuries, traumatic brain injury is a major cause of disability and death, particularly in young active individuals. While IF has not been evaluated in animals models of traumatic brain injury, it was reported that CR (limited daily feeding with a 30% reduction in calorie intake) initiated 4 months prior to the injury, reduced the extent of brain damage, ameliorated cognitive deficits, and elevated BDNF levels in the affected cerebral cortex and hippocampus. Finally, recent studies have elucidated the potential impact of IF on peripheral nerve health and disease resistance. In a mouse model of the peripheral demyelinating neuropathic disease Charcot-Marie-tooth type 1A (Trembler mice), 5 months of ADF resulted in improved motor performance, increased myelination and decreased accumulation of PMP22 protein aggregates. Additional findings suggest that the beneficial effects of IF on peripheral nerve health and disease resistance are mediated, in part, by up-regulation of autophagy and related protein quality control mechanisms.[27]

2.7 Intermittent fasting and cancer

Recently a series of studies in animal models have shown that periodic fasting (PF) lasting 2 or more days can be as effective as chemotherapy in delaying the progression of a wide range of cancers but, more importantly, can protect normal cells from the toxic effects of chemotherapy drugs while sensitizing cancer cells to the treatment. A severely restricted diet that mimics PF started at middle age was effective in causing a major reduction in tumor incidence, in addition to delaying tumor onset and reducing the number of sites with tumor-like lesions, suggesting a reduction in metastatic cancers. The role of PF and FMDs in cancer prevention and treatment has been discussed in more detail elsewhere.

IF has been studied in murine cancer models, although mostly in cancer prevention. They studied the effects of ADF on the survival of 3–4 month-old tumor-free and tumor-bearing Fisher rats. 50% of

the ADF rats survived to day 10 compared to 12.5% survival in the control diet group. In addition, this study included both a tumor prevention and a tumor treatment component since the ADF was initiated one week before rats were inoculated intraperitoneally with ascites tumor cells, making it difficult to understand the mechanisms responsible for its effects.[27]

2.7.1 IF and cancer in humans:

Adipose tissue shows increased leptin production and decreased adiponectin production as adiposity increases, which is believed to play a role in cancer development and progression through effects on insulin sensitivity, inflammation and direct effects on cell proliferation and apoptosis. Adiponectin levels increase in overweight people only after CER, when there is a large reduction in body and visceral fat (>10%). Some IF studies have reported an increase in adiponectin with more modest weight loss, i.e. a 30% increase in plasma adiponectin on both restriction and feeding days, along with modest reductions in weight (-4%) and body fat (-11%). There was a tendency for a greater increase in adiponectin with IF than with CER, despite a comparable reduction in weight and adiposity. IF results in large and comparable reductions in leptin compared to CER (both 40%) and in the leptin/adiponectin ratio. Weight loss with CER reduces circulating levels of C-reactive protein (CRP) by 2-3% for every 1% weight loss, while TNF-α and IL-6 are reduced by approximately 1-2% for every 1% weight loss. The reductions in inflammatory markers with IF are comparable to those of CER for a given weight loss. Therefore, although limited, the available biomarker data suggest that IF leads to changes in most cancer risk biomarkers comparable to those of CER, with the possible exceptions of insulin resistance and adiponectin, which require further studies with robust methodologies.[27]

There are no data on the effects of FH on cancer rates in humans. Weight control is likely to reduce the risk of thirteen cancers that

have been linked to obesity, although the role of weight management after diagnosis on the outcome of obesity-related cancers is unknown. Surrogate evidence that FI can reduce cancer risk may be derived from its effects on a number of biomarkers of cancer risk, such as insulin, cytokines and inflammation-related molecules, leptin and adiponectin, which are believed to mediate the effects of adiposity and excessive energy intake on tumor development and growth in humans.[27]

The effect of FI on total and bioavailable insulin-like growth factor 1 (IGF-1) in human studies has been variable. This reflects the fact that, unlike in animal studies, circulating levels of total IGF-1 and bioactive IGF-1 (determined by measuring IGF-binding proteins 1,2 and 3) are poor markers of the effects of energy restriction and weight loss in humans and do not correlate well with IGF-1 bioactivity at the tissue level. Changes in total circulating IGF-1 concomitant with weight loss with IF or CER have been reported in various studies. IF and CER both increased IGF-binding protein-1 (26% and 28%) and IGFBP-2 (22% and 36%) but did not change bioavailable IGF-1 in serum (ultrafiltrate) when measured after days of feeding. There was a further acute 17% increase in IGF-binding protein-2 in the morning after the two-day restriction of an ER to 70%, but no measurable change in total or bioavailable IGF-1 in serum (ultrafiltrate). Reductions in IGF-1 (-15%) were reported in normal and overweight subjects who followed an IF diet that included 5 days per month of a low-protein, low-energy diet (~0.25g protein/kg weight, 34-54% of normal energy intake) interspersed with a normal intake for the remaining 25 days of the month. These reductions were observed after 5 days of normal feeding after three months, together with modest reductions in body weight (-2%). The aforementioned effects of FH in relation to insulin resistance and diabetes risk could therefore play an important role in protecting against obesity-related cancers.[27]

3 Study

3.1 Aims

Given the paucity of data available, the primary objective of the present study is to evaluate the safety and tolerability of three cycles of a 7 days FMD as a potential support to the management of patients affected by RRMS, already treated with first-line therapies (interferon-beta-1a/b, galatiramer acetate, teriflunomide and dimethyl fumarate). These treatments are among the most used treatments in MS with a well-known and characterized safety and tolerability profile and represent an ideal backbone therapy for an association with a FMD.

Secondary objectives are:

- Assess the patients' compliance to the FMD;
- Determine if a FMD has an effect on general health status, including nutritional status and body composition;
- Evaluate if a FMD has some effect on the neurological clinical status of the patient;
- Evaluate if a FMD has some influence on the inflammatory activity of the disease as evaluated by MRI;

3.2 Study Endpoints

The primary endpoint is the number of serious and/or severe adverse events after the FMD start. Adverse events (AE), Adverse drug reactions (ADR), Serious adverse events (SAE) and Suspected unexpected serious adverse reactions (SUSAR) were carefully collected and registered in an electronic case report form (eCRF) and documented in the study report.

Secondary endpoints are:

- Assessment of patients' compliance by phone interviews by a

nutritionist at day 1 and 5 of every FMD cycle;
- Changes in the composition in blood samples collected at the same time-points;
- Changes over the follow-up period in BMI and body composition as assessed by a dynamometer and by BIA technology at baseline and at month 2, 4 and 6;
- Changes over the follow-up period in the EDSS scores, evaluated at baseline and month 6;
- Number of relapses over 6 months;
- Changes over the follow-up period in the number of new T2/FLAIR and T1-Gd+ lesions on MRI scan at baseline and month 6;

3.3 Methods

This study is an open-label, single-arm, run-in study in patients with RRMS treated with first line therapies (interferon-beta-1a/b, glatiramer acetate, teriflunomide and dimethyl fumarate), assessing the feasibility and tolerability of 3 cycles of FMD over 6 months. The overall study duration is 9 months.
In this ongoing, phase-II prospective study, we enrolled 24 consecutive RRMS patients followed at the MS Center of the University of Genoa undergoing first-line therapies.

Inclusion criteria of the study are:
- Diagnosis of RRMS[7];
- Age 18 to 50 years;
- Disease duration 6 months to 10 years (included);
- EDSS 0 to 4, 5;
- Treatment with first line therapies (interferon-beta-1a/b, glatiramer acetate, teriflunomide and dimethyl fumarate).

Exclusion Criteria of the study are:
- < 6 as months since treatment start with first line therapies (interferon-beta-1a/b, glatiramer acetate, teriflunomide and dimethyl

fumarate);
- Relapse < 60 days;
- Any active or chronic infection;
- Previous history of a malignancy other than basal cell carcinoma of the skin or carcinoma in situ that has been in remission for more than one year;
- Severely limited life expectancy by another co-morbid illness;
- Nutritional risk screening (NRS 2002) > or = 3;
- History of previous diagnosis of myelodysplasia or previous hematologic disease or current clinically relevant abnormalities of white blood cell counts;
- Pregnancy or risk or pregnancy (this includes patients that are unwilling to practice active contraception during the duration of the study);
- e-GFR < 60 mL/min/1.73m2 or known renal failure or inability to undergo MRI examination;
- Inability to give written informed consent in accordance with research ethics board guidelines;
- Known alimentary allergy or intolerance to any of the ingredients of the FMD regimen.

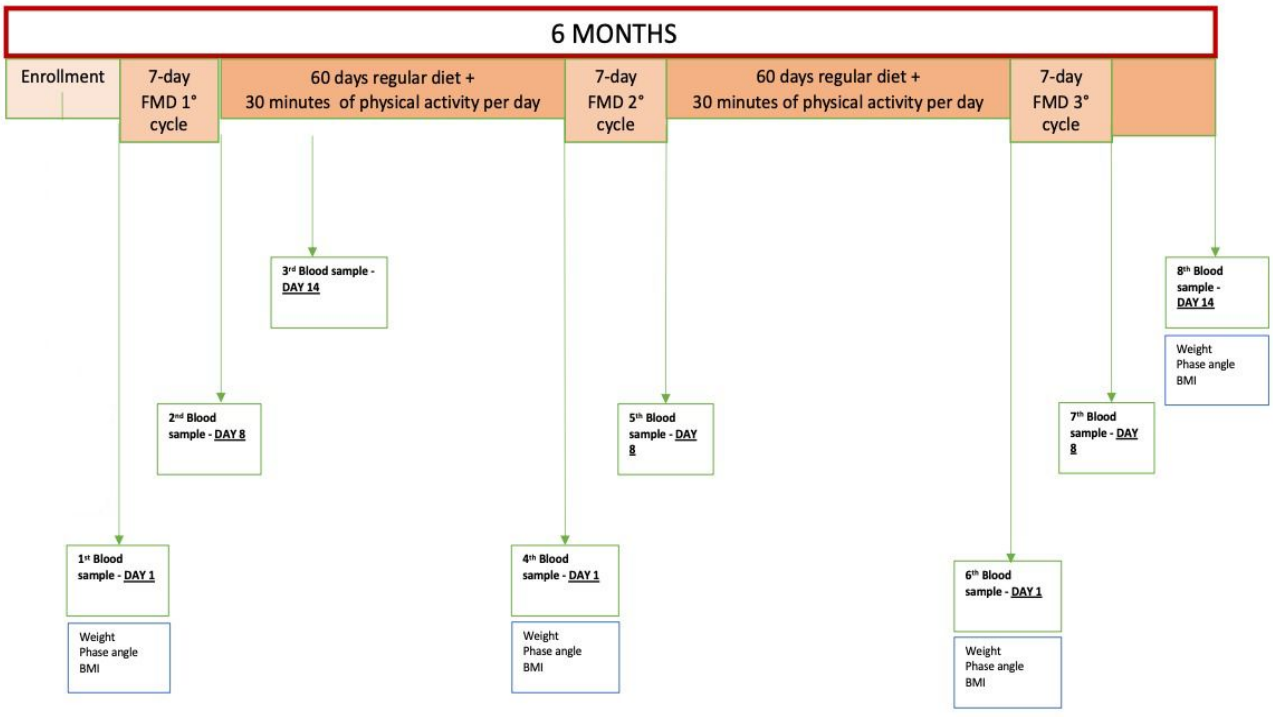
3.4 FMD

Patients were asked to undergo 3 cycles of 7-days FMD, that provides 1100 kcal on day one and 800 kcal on days 2-7 every 60 days in addition to standard therapy. The diet consists of specific food items and dietary supplements selected for their fasting-mimicking properties, which are Generally Regarded As Safe (GRAS). The following components were included:

- Vegetable soups: Tomato, spinach, mushroom, pumpkins, and other varieties;
- Energy bars;
- Snacks: Kale chips, dried/baked vegetable chips, olives, and similar options;

- Energy drinks;
- Teas;
- Algal oil softgels: Softgel capsules containing omega-3 essential fatty acids;
- Dietary supplement pill: A pill containing a proprietary blend of vitamins and minerals, including Vitamin A, Vitamin C, Vitamin D, Vitamin E, Vitamin K, Thiamine, Riboflavin, Niacin, Vitamin B6, Folic Acid, Vitamin B12, Biotin, Pantothenic Acid, Calcium, Iron, Phosphorous, Iodine, Magnesium, Zinc, Selenium, Copper, Manganese, Chromium, and Molybdenum. The pill also contains a proprietary blend of beet root powder, spinach leaf powder, tomato fruit powder, carrot root powder, collard greens powder, and kale leaf powder, along with other inactive ingredients.

Alcohol and other energy drinks were not permitted.



Tab.5: Time of enrollment.

A nutritionist followed the patients for the entire 6 months, with the aim of monitoring the effects of the FMD and checking for any problems. During days of calorie restriction patients were called by

the nutritionist on the first day of each FMD cycle to answer any questions and check the response, and on the fifth day of FMD to check side effects and compliance.

During the scheduled visits, each patient has vital signs and adverse events recorded. Blood samples and anthropomorphic parameters including BMI, weight, and phase-angle (PHA) were collected at baseline and shortly before day 7, before return to normal diet in cycle 1 and one week after cycle 1, 2 and 3 of FMD (see table 5). The blood test consists of a complete metabolic profile with red (RBC) and white blood cells (WBC), glucose, BUN, creatinine, liver enzymes, sodium, potassium, ketonuria, proteinemia, and urine evaluation. If the patient shows abnormal values, the blood count is repeated one week later. No further cycles of FMD were allowed in patients who do not return to normal white blood cell (WBC) and red blood cell (RBC) levels.

Adverse events (AEs) were monitored for each patient until the end of the study; in case of ongoing AEs at the end of the study, patients were followed up for another 30 days.

Changes terms of general health status, including disability and MRI activity were determined at baseline and after month 6.

3.5 Statistical Analysis

SPSS 23 (IBM) was used for computation. Descriptive results were reported as mean with standard deviation (SD) or median with interquartile range (IQR). Descriptive statistical methods were used for the analysis of the primary endpoint. In particular, serious and/or severe adverse events after the FMD start were summarized descriptively by mean of frequency. The number of patients who did not tolerate the FMD was reported as a proportion over the total. Differences in terms of the explored variables before and after the cycles were assessed with repeated measure analysis of variance (ANOVA). A t-test was used to explore differences in terms of baseline values of glucose between patients who presented fatigue

during each cycle. A p-value < 0.05 was considered statistically significant.

4 Results

4.1 Population characteristics

Of the 24 patients initially enrolled 2 of withdrew consent before starting the diet. Accordingly, we included N=22 patients in the final analyses [female: 63,6%; mean (SD) age and disease duration: 44 (6.8) and 11,6 (6.9); median (range) EDSS: 1 (0-2,5)]. Population characteristics are reported in Table 6.

Demographic data	N=22
Mean (SD) age y	44 (6,8)
Female, n (%)	14 (63,6)
Median (range) baseline EDSS	1 (0-2,5)
Mean (SD) disease duration, y	11,6 (6,9)
Treatment, n	
-interferon-beta	5
-galatiramer acetate	2
-teriflunomide	3
-dimethyl fumarate	12
Completed 1 cycles, n (%)	22 (100)
Completed 2 cycles, n (%)	20 (91)*
Completed 3 cycles, n (%)	17 (77)**

** 2 patients have not completed the third cycle*

*** 3 patients dropped after the 1st cycle due to non adherence*

Tab.6: Population characteristics.

4.2 Primary outcomes

4.2.1 Adverse events:

According to the Common Terminology Criteria for Adverse Events (CTCAE), 22 patients reported after the first cycle of FMD, grade 1 fatigue (N=13), headache grade 1 (N=8) and grade 2 (N=3), vomiting (N=1), sleepiness (N=3), nausea (N=5), hypotension (N=1), depression (N=2), chills (N=2), constipation (N=2), back pain (N=1), diarrhea (N=2), tachycardia (N=2), generalized muscle weakness (N=1) and fever (N=1) and grade 2 impaired concentration (N=1) and presyncope (N=1).

After the second cycle of FMD, 20 patients reported grade 1 fatigue (N=15), headache grade 1 (N=10) and grade 2 (N=1), vomiting (N=2), sleepiness (N=2), insomnia (N=1), nausea (N=3), hypotension (N=1), depression (N=1), chills (N=1), tachycardia (N=1), stomachace (N=1) and fever (N=1) and grade 2 impaired concentration (N=1).

Of the 17 patients who completed the third cycle, they reported grade 1 fatigue (N=7), headache (N=7), vomiting (N=2), sleepiness (N=1), nausea (N=2) and grade 2 impaired concentration (N=1) (see table 7).

Adverse events	1st cycle N=22	2nd cycle N=20	3rd cycle N=17
Fatigue	13 (G1)	15 (G1)	7 (G1)
Headache	8 (G1), 3 (G2)	10 (G1), 1 (G2)	7 (G1)
Nausea	5 (G1)	3 (G1)	2 (G1)
Sleepiness	3 (G1)	2 (G1)	1 (G1)
Vomiting	1 (G1)	2 (G1)	2 (G1)
Depression	2 (G1)	1 (G1)	0
Hypotension	1 (G1)	1 (G1)	0
Chills	2 (G1)	1 (G1)	0
Tachycardia	2 (G1)	1 (G1)	0
Constipation	2 (G1)	0	0
Back pain	1 (G1)	0	0
Diarrhea	2 (G1)	0	0
Generalized Muscle Weakness	1 (G1)	0	0
Stomachache	0	1 (G1)	0

Impaired concentration	1 (G2)	1 (G1)	1 (G1)
Presyncope	1 (G2)	0	0
Fever	1	1	0
Insomnia	0	1	0

Tab.7: Advers events after 1st,2nd and 3rd cycle.

4.3 Secondary outcomes

4.3 Diet adherence:

The nutritionist has phoned patients on the first day of each FMD and on day 5 of each FMD, in order to respectively support the patient with any doubt or question on the diet to follow and to check side effects and diet compliance. During the course of the study, 3 patients dropped out after completing the first cycle due to non-adherence and 2 patients have not yet completed the third cycle.

4.2.2 Differences between blood test after every cycle:

Blood samples have been collected at baseline, immediately after the first cycle and 5-7 days after the end of the third cycle of the FMD. For analyses regarding blood test samples, we included only the 17 patients who completed the 3 cycles.

Considering the diagnosis of hypoglycemia, defined as glucose values < 70 mg/dl[29], after the first diet cycle, 4 patients had blood glucose values < 70 mg/dL and 1 patient had values<65 mg/dL. After the second cycle, 2 patients had glucose values <65mg/dL. After the third cycle, 4 patients reported blood glucose values <70 mg/dL and 1 patient reported values <65 mg/dL.

All other blood test values remained within the normal limits at each time point.

A mild increase in aspartate-aminotransferase (AST) levels (see Fig. 3) and in bilirubin levels (Fig. 4) after each cycle, with a return to

baseline values after 7 days, emerged (ANOVA analysis: $p<0.05$ for each variable).

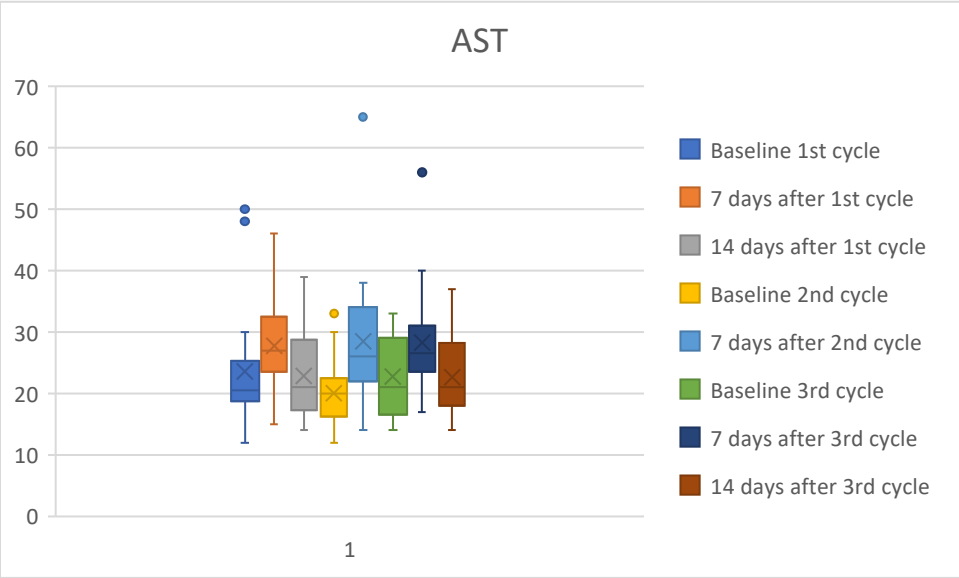


Fig.3: AST trend.

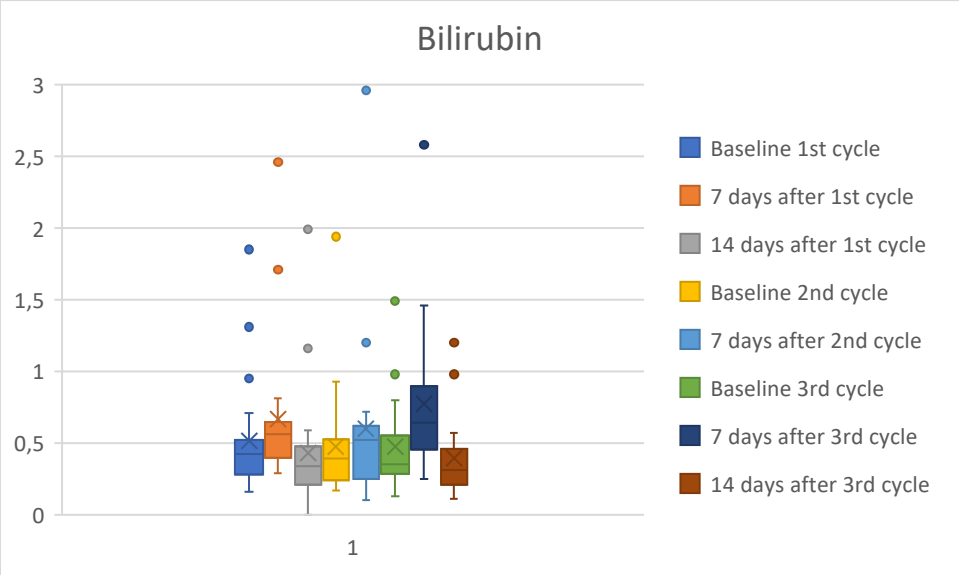


Fig.4: Bilirubin trend.

Similarly, gamma-glutamyltransferase (GGT) values decreased after the second and third cycles (see Fig. 5), with a return to baseline values after 7 days (ANOVA analysis: $p<0.05$).

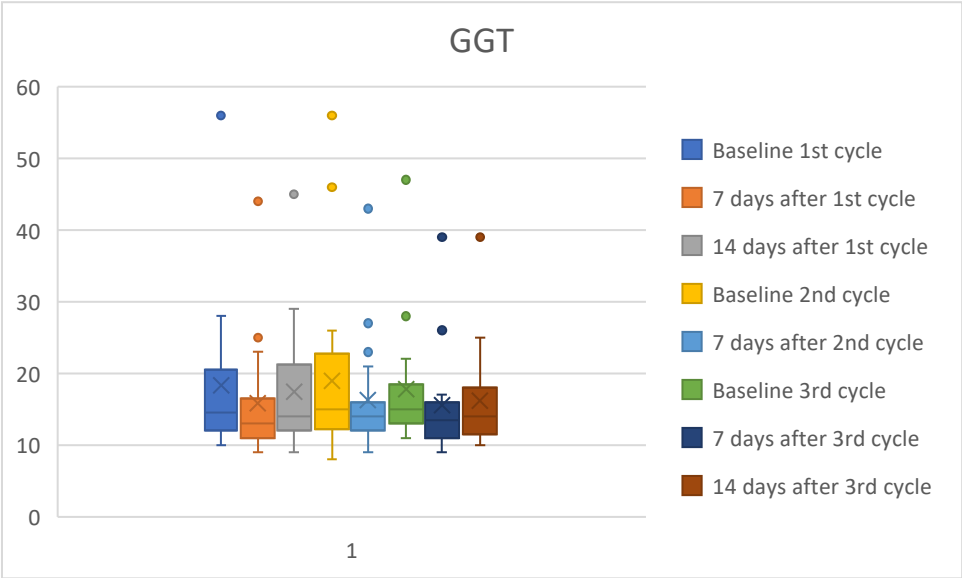


Fig.5: GGT trend.

Importantly, the values of total WBC and lymphocytes (Fig. 6) did not show any significant changes after the 3 cycles of FMD.

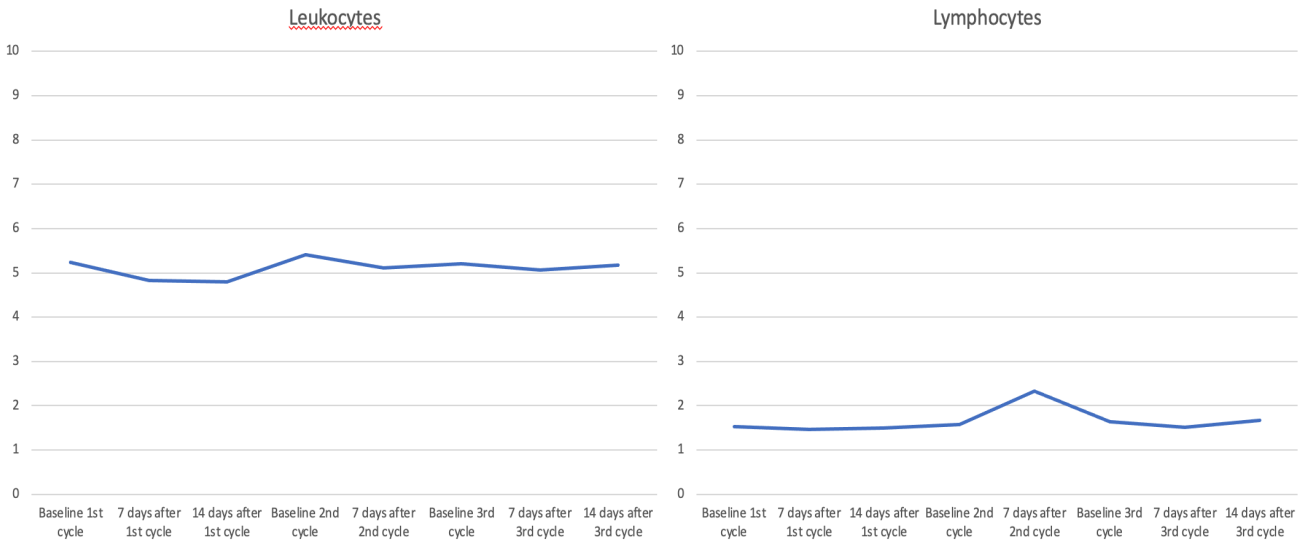


Fig.6: Leukocytes and lymphocytes after the 3 cycles.

Since blood glucose was the only parameter that moved beyond the normal range, we wanted to investigate if values differed in patients with fatigue (the most frequent AE observed) and found that blood glucose values were significantly lower at baseline in the first and second cycles in patients who presented with fatigue during the cycle of diet. As shown in Figure 7, we observed that baseline values of glucose were statistically significantly lower in patients who reported fatigue during the first and second cycles as compared to patients who did not report this AE (p=0.03 and p=0.02, respectively). This difference did not reach statistical significance during the third cycle (p=0.07).

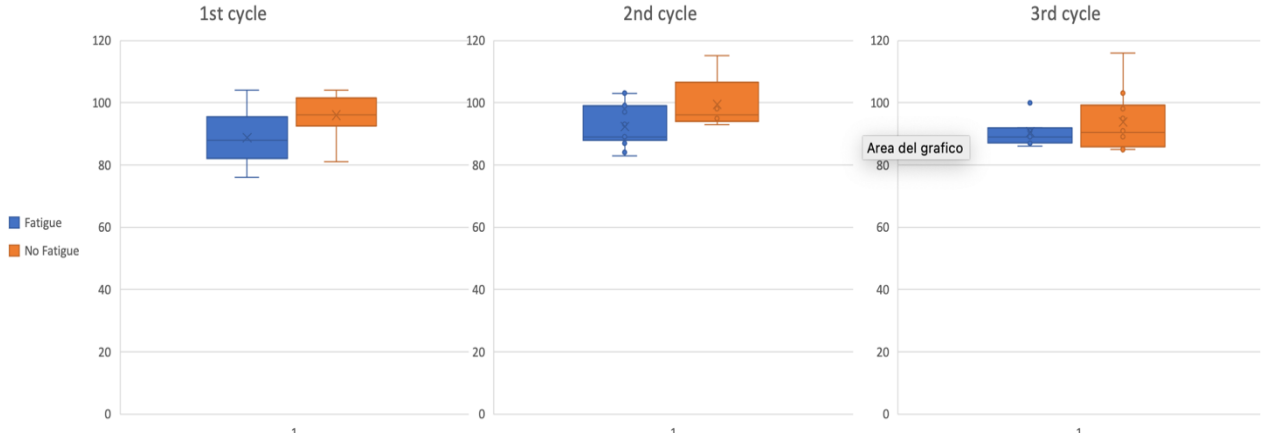


Fig.7: Baseline glucose values in 1st, 2nd and 3rd cycles in patients who reported fatigue.

4.4 Change in weight, BMI and phase angle:

We analyzed the complete data of the 17 patients who completed the third cycle and found that BMI values ranged from 21 to 46. The average BMI at baseline was 26.7 and it slightly decreased to 26.1 at the end of the third cycle.

Regarding weight, the minimum weight was 53 kg, and the maximum weight was 128 kg. The mean weight at baseline was 77 kg, and after the first cycle, it decreased to 76.3 kg. It further decreased to 75.0 kg after the third cycle.

The phase angle, which ranged from 5.4 to 6.9, had a mean value of

5.9 at baseline. After the first cycle, the mean value slightly decreased to 5.8 and then returned to 5.9 after the third cycle.
At repeated measure ANOVA analysis, none of these differences resulted statistically significant ($p>0.05$ for each comparison).

	BMI N=17	Weight N=17	Phase Angle N=17
Baseline	26.7	77.0	5.9
1st cycle	26.5	76.4	5.8
2nd cycle	26.5	76.3	5.9
3rd cycle	26.1	75.0	5.9

Tab.8: Mean Value of BMI, weight and phase angle.

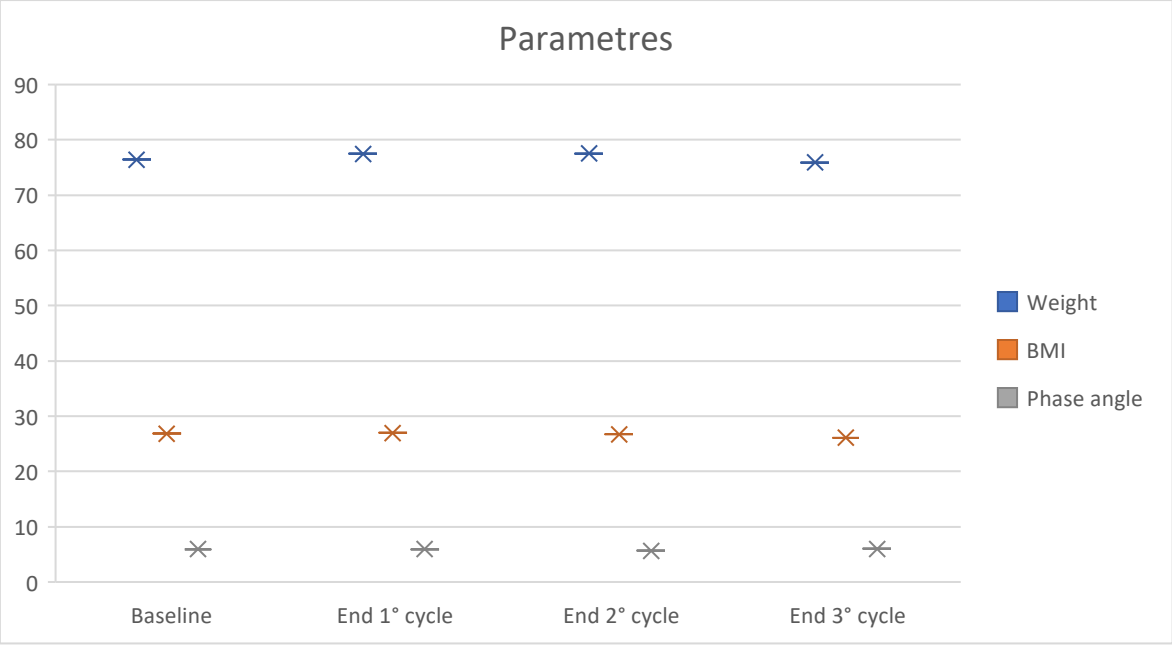


Fig.6: Weight, BMI and phase angle during the three cycles.

4.5 Impact of FMD in terms of clinical and radiological MS outcomes:

In the 17 patients who completed the study, no changes were observed in terms of disability status, relapses, or MRI activity.

5 Discussion

In this study we aimed to investigate the impact of FMD on patients with RRMS. Scientific interest about diet and its potential to impact the course in MS, has grown significantly in the last few years. Given the critical need for more definitive evidence linking specific diets to clinical outcomes, we aimed to evaluate the safety and tolerability of FMD among people with RRMS.

Generally, the diet was well- tolerated by the participants, and only a few adverse events were reported (see table 7). The most frequent adverse event reported by participants in this study was fatigue. We observed only moderate symptoms grade 1 and 2 severity together with an overall stability in terms of body composition, nutritional status and blood tests.

Adherence to the diet interventions, regardless of the intensity of clinical support provided to participants, was good but it may represent a barrier to the feasibility of large studies.

Change in weight over 6 months, a more objective measure of diet adherence, similarly demonstrated no meaningful weight loss over the study period.

Among the 17 patients who completed the third cycle, no significant changes were observed in terms of phase angle and BMI. This indicates that the FMD did not have a significant impact on these measures.

In none of the evaluated patients showed changes in their EDSS scores, experienced disease relapse, or exhibited radiological disease activity. However, since there was no comparison group in the study, it is not possible to determine whether these outcomes were solely due to the impact of the diet.

Importantly, the blood test results showed no lymphopenic effects, despite participants taking lymphopenizing drugs as part of their MS treatment. This suggests that the FMD did not negatively affect lymphocyte levels in these individuals.

6 Conclusion

These results provide evidence that fasting mimicking diet is safe and well tolerated and resulted in acceptable compliance among people with RRMS. However, large, randomized controlled studies are still needed to provide strong evidence regarding whether dietary modification in people with MS is of clinical benefit.

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