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Dietary restriction in multiple sclerosis: evidence from preclinical and clinical studies

Abstract

Dietary restriction (DR) interventions, which encompass both chronic and intermittent reductions in energy intake, are emerging as potential therapeutic approaches for dampening neuroinflammation and demyelination in multiple sclerosis (MS). Mechanisms mediating the beneficial effects of DR include the regulation of pro- and anti-inflammatory signalling molecules and gut microbiome remodeling. This article summarises the preclinical evidence supporting the role of DR in attenuating disease in animal models of MS and the developing clinical evidence indicating the safety and feasibility of such DR interventions in people with MS (pwMS).

of reactive oxygen species by adipose tissue macrophages [10-11]. Furthermore, higher adiposity is associated with increased serum levels of leptin, a pro-inflammatory adipokine [12]. This chronic inflammatory state may act to predispose overweight and obese individuals to the development of autoimmunity. Moreover, obesity is linked to dysbiotic alterations of the gut microbiota [13-14]. The gut microbiota is emerging as a potential modulator of pathogenic immune responses, with animal studies providing evidence for the role of gut microbiota in regulating T lymphocyte differentiation, CNS inflammation and microglia function, as well as myelination and blood-brain barrier integrity [15-18]. Correspondingly, people with MS (pwMS) display moderate gut microbiota dysbiosis [19,20].

Dietary restriction (DR) without malnutrition is a powerful intervention shown to extend healthy lifespan in many animal species, including non-human primates [21]. Furthermore, DR promotes weight loss and reduces multiple markers of inflammation in humans and also the experimental autoimmune encephalomyelitis (EAE) model of MS [22-27]. DR also prevents demyelination and promotes remyelination in toxin-induced models of MS [26,28-30]. In this article, we will review the beneficial effects of DR, including its ability to lower levels of pro-inflammatory molecules and reshape gut microbiome composition, highlighting the potential utility of DR for protecting against neuroinflammation and demyelination in pwMS.

Dietary restriction

Dietary restriction (DR) is defined here as a reduction in total food intake, either chronically or intermittently, whilst maintaining proper nutrition. Chronic DR, also known as calorie restriction (CR), entails a variable reduction in energy intake every day (usually ~20% in humans and up to 40-50% in preclinical models), wherein meal frequency remains unchanged. Intermittent fasting (IF) involves complete abstinence or substantial reduction of energy intake for periods of time, usually

Multiple sclerosis (MS) is considered an autoimmune disease of the central nervous system (CNS), resulting from the complex interplay between genetic and environmental risk factors. Several epidemiological studies conducted over the last decade have established the association between early-life obesity and elevated future risk of MS development [1]. Overweight and obesity (BMI ≥ 25 or 30, respectively) in adolescents and young adults increased MS risk by two-fold [2-5]. Mendelian randomisation studies have correlated genetic determinants of high BMI with heightened MS susceptibility, accounting for possible confounding lifestyle and socio-economic factors and supporting a causal relationship between obesity and MS [6-7]. Moreover, obesity was associated with an almost two-fold increased relapse risk, greater annual increase in disability [8], and higher brain volume loss [9].

Multiple mechanisms may underlie the obesity-mediated increase of MS risk, some of which are not clearly understood and are the subject of ongoing investigations. Firstly, obesity is characterised by chronic low-grade inflammation with increased secretion of inflammatory mediators including IL-6, IL-12 and TNF- α and enhanced production

12 hours or longer, and unrestricted feeding during meal times [31]. Examples of IF regimens include time-restricted feeding (TRF), in which total daily food intake is limited to a specific timeframe within the day (typically lasting between 6-8 hours), fasting or drastic caloric reduction (e.g., consumption of 500 calories per day) on alternate days (alternate day fasting or alternate day modified fasting), fasting for 2 days per week (5:2 diet), and fasting mimicking diet (FMD) which comprises several days (usually 5-7 days in humans or 3 days in preclinical models) of drastically reduced calorie intake [32]. Collectively, DR interventions can induce healthy weight loss in overweight individuals and more importantly, exert beneficial anti-inflammatory and neuroprotective effects both in relation to and independent of reduced adiposity [24].

Balancing the pro-inflammatory versus anti-inflammatory cytokine milieu

The adipose tissue serves as not only an energy reservoir, but also an endocrine organ, secreting cytokines and hormones, collectively termed “adipokines”, which can modulate immune responses [33]. A standard Western diet (WD) is characterised by the regular intake of high amounts of processed foods, red meat, high-fat dairy products, high-sugar, and pre-packaged foods and leads to excess adiposity with consequent adipokine dysregulation characterised by upregulation of pro-inflammatory molecules, such as leptin, C-reactive protein, TNF α and IL-6, and down-regulation of anti-inflammatory factors, such as adiponectin [34] (Figure 1). CR and IF interventions reduce visceral adiposity in humans [35,36] and restore the balance between pro-inflammatory and anti-inflammatory mediators in animals and humans [22,37,38]. DR reliably decreases leptin levels and increases adiponectin levels in animals and humans [24,39,40] (Figure 1). Pertinently, pwMS display elevated leptin and reduced adiponectin levels, with leptin concentration correlating negatively with the number of regulatory T cells [41,42]. Hence, DR restriction may be an effective tool for tempering the dysregulated cytokine milieu in MS (Figure 1).

Gut microbiome and neuroinflammation

A considerable number of studies have reported alterations in the gut microbiome composition in pwMS. Unfortunately, there is little overlap in results between studies and only some taxa are consistently reported as differentially represented. Several studies reported an over-representation of the genera *Akkermansia* in pwMS compared to healthy controls [15,19,43] together with a reduced abundance of *Bacteroidaceae*, *Faecalibacterium*, *Clostridium* species and *Prevotella* strains [44-46]. Both *Clostridium* and *Bacteroidaceae* have immunomodulatory effects, promoting the differentiation of murine regulatory T cells and the production of IL-10 [47-49] (Figure 1). It is unclear whether these gut-microbiota alterations in MS are a consequence of the disease or

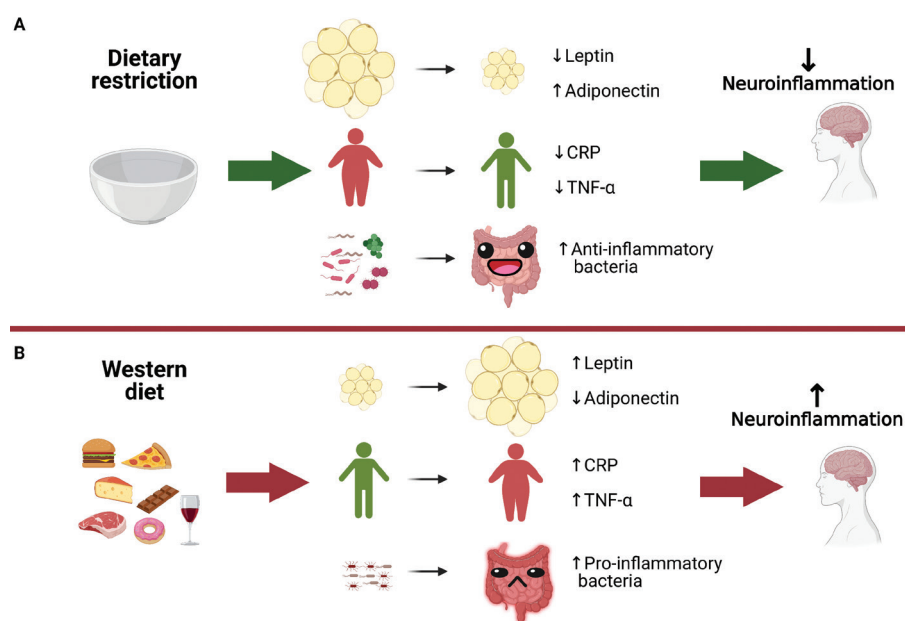


Figure 1. Main effects of DR compared to a Western diet regimen. A. Lowering caloric intake determines a reduction in adiposity, with a consequent decrease in leptin and increase in adiponectin, lower levels of systemic inflammation and a modulatory effect on the gut microbiome. B. On the contrary a Western diet (WD) regimen increases adiposity, the production of pro-inflammatory cytokines and adipokines and the abundance of pro-inflammatory gut bacteria. These diet-induced systemic changes will impact neuroinflammation.

contribute to disease pathogenesis. However, in support of a real pathogenic role is the finding that gut microbiota or gut-derived molecules obtained from pwMS can modulate disease in the main MS animal model, EAE, when transferred into mice [15,43]. Specifically, recipient mice inoculated with microbiota from pwMS showed increased incidence of spontaneous EAE [43] and exacerbated EAE clinical severity with decreased regulatory T cell expression compared to those inoculated with healthy control microbiota [15]. Furthermore, extracts from specific MS-associated bacterial species increased in vitro differentiation of pathogenic CD4⁺ T helper 1 cells, whilst extracts from bacterial species that were diminished in pwMS boosted regulatory T cell differentiation [15]. These findings indicate a role for MS-related microbiota in shaping immune phenotypes and function.

DR interventions have the potential to positively influence gut microbiota composition. For example, life-long calorie restriction changed gut microbiota structure in mice, as marked by an enrichment of anti-inflammatory bacterial strains including *Lactobacilli* [50]. Microbial alterations were accompanied by reductions in serum levels of lipopolysaccharide-binding protein, a marker of gut-derived endotoxin load [50], which is also correlated with systemic inflammation.

Search strategy

To find studies testing DR regimens in preclinical models of MS and pwMS, a literature search was conducted in PubMed in September 2021 using the search string ((multiple sclerosis) OR (CNS autoimmunity) OR (experimental autoimmune encephalomyelitis) OR (experimental allergic encephalomyelitis)) AND ((dietary

restriction) OR (calorie restriction) OR (caloric restriction) OR (fasting) OR (intermittent)). After reading titles and abstracts, only original research articles investigating restriction of total food intake (not specific macronutrients or micronutrients) were included.

Effects of dietary restriction in preclinical models

There is strong preclinical evidence supporting the efficacy of DR regimens, including both CR and IF, in protecting against neuroinflammation and demyelination (Table 1). The preventative effects of CR in EAE were first demonstrated in 2004 [51]. Prior to immunisation, Lewis rats were subjected to 15 days of severe CR, equivalent to 66% reduction from ad libitum intake and then monitored for EAE symptoms. Whilst 8 out of 9 ad libitum-fed rats developed clinical signs of EAE, calorie-restricted rats did not display any disease manifestations and demonstrated reduced lymphocyte response to T-cell mitogen concanavalin A [51]. A follow-up study utilising the same 15-day CR protocol revealed that moderate CR (33% reduction) was insufficient for preventing EAE progression, whilst severe 66% CR-induced inhibition of EAE development was likely mediated by decreased IFN- γ production [52]. Our group demonstrated that a month of 40% CR in mice delayed EAE clinical onset and decreased disease severity [23]. CR was associated with increased levels of adiponectin and corticosterone, and decreased levels of IL-6 and leptin [23].

IF administered before immunisation and throughout the course of EAE similarly delayed disease onset and reduced the incidence and severity of disease [24,53]. Along with attenu-

Table 1. Summary of the studies that tested DR regimens in MS animal models

Type of DR	DR effects on inflammatory or other brain pathology markers	DR effects	Reference
66% CR for 15 days before EAE induction and throughout disease course	↓ lymphoid cell mitogenic response to concanavalin A	Prevents EAE	[51]
66% CR for 15 days before EAE induction and throughout disease course	Alters lymphocyte composition in lymphoid organs, ↓ IFN- γ production	Prevents EAE	[52]
40% CR for 5 weeks before EAE induction and throughout disease course	↑ corticosterone and adiponectin, ↓ leptin	Ameliorates EAE clinical course	[23]
IF – alternate-day fasting for 8 weeks before EAE induction and throughout disease course	Not reported	Ameliorates EAE clinical course and reduces incidence of disease	[53]
IF – alternate-day fasting for 4 weeks before EAE induction and throughout disease course	↓ Th17 cells, ↑ T regulatory cells in small intestine lamina propria	Ameliorates EAE clinical course and reduces incidence of disease	[24]
IF – alternate-day fasting for 4 weeks before EAE induction and throughout disease course	↓ monocyte infiltration in the spinal cord, ↓ monocyte expression of TNF α , IL-1 β , CXCL2 and CXCL10	Ameliorates EAE clinical course and reduces incidence of disease	[25]
FMD – 3 cycles of very-low-calorie and low-protein diet for 3 days per week after EAE induction	↓ immune cell infiltration in the spinal cord, ↑ autoreactive lymphocyte apoptosis, ↑ oligodendrocyte differentiation	Ameliorates EAE clinical course and reduces incidence of disease	[26]
FMD – 2 cycles of 67% calorie reduction for 3 days per week after EAE induction	↓ immune cell infiltration in the spinal cord, ↑ oligodendrocyte precursor cells in the spinal cord, ↑ BDNF expression	Ameliorates EAE clinical course and reduces incidence of disease	[27]
33% CR for 4 weeks	↑ remyelination, ↑ mRNA expression of BDNF, ↓ astrogliosis and microgliosis, ↑ oligodendrogenesis	Ameliorates cuprizone-induced demyelination	[28]
CR via 10% cellulose diet for 6 weeks	↓ demyelination, ↓ M1 iNOS ⁺ macrophage/microglia, ↑ M2 arginase 1 ⁺ macrophage/microglia, ↓ corpus callosum TNF α expression	Ameliorates cuprizone-induced demyelination	[29]
IF – alternate-day fasting for 6 months	↑ remyelination after induced focal demyelination, ↑ oligodendrocyte progenitor cell differentiation	Enhances remyelination in aged rats	[30]

Abbreviations: ↑, increase; ↓, decrease; BDNF, brain-derived neurotrophic factor; CR, calorie restriction; CXCL2, chemokine (C-X-C motif) ligand 2; CXCL10, chemokine (C-X-C motif) ligand 10; DR, dietary restriction; EAE, experimental autoimmune encephalomyelitis; FMD, fasting mimicking diet; IF, intermittent fasting; IFN- γ , interferon gamma; IL-1 β , interleukin 1 beta; iNOS, inducible nitric oxide synthase; mRNA, messenger ribonucleic acid; Th17, T-helper 17; TNF α , tumour necrosis factor alpha.

ation of EAE, IF decreased Th17 cells and increased regulatory T cells in the small intestine lamina propria and also increased the diversity and altered the composition of the gut microbiome [24]. Notably, gut microbiota transfer from IF mice to naïve EAE recipients recapitulated the protective effects of IF, suggesting the role of microbiota in mediating the favorable effects of IF [24]. Another study found that IF conferred protection against EAE by preventing monocyte recruitment to the CNS and downregulating monocyte expression of pro-inflammatory genes including TNF α , IL-1 β , CXCL2 and CXCL10 [25]. Importantly, IF did not compromise the immune response to bacterial infection or tissue injury [25]. FMD, a form of IF applied in cycles which consist of several days of severe reduction in calorie intake followed by ad libitum feeding, has also been effective in ameliorating EAE [26,27]. FMD administered therapeutically after disease onset suppressed autoimmunity as evidenced by reduced pro-inflammatory cytokines, Th1 and Th17 cells and antigen-presenting cells and promoted recovery as demonstrated by enhanced oligodendrocyte regeneration,

remyelination [26], and brain-derived neurotrophic factor (BDNF) expression [27].

The beneficial effects of DR have also been reproduced in preclinical models of demyelination and remyelination [26,28-30]. In the cuprizone model (in which oligodendrocyte death is caused by administration of a toxin), 33% DR improved coordination and balance and enhanced corpus callosum remyelination. DR reduced astrogliosis and microgliosis and expanded the oligodendrocyte population [28]. Further, DR skewed macrophage/microglia polarisation towards the anti-inflammatory M2 phenotype in the cuprizone model [29]. Macrophage/microglia phenotypic switching may be modulated by activation of nutrient sensors and modulation of immunometabolic pathways [54,55]. In another study, 6 months of alternate-day fasting increased remyelination in aged rats after focal demyelination induced by ethidium bromide injection through restoring the regenerative capacity of oligodendrocyte precursors [30]. Overall, preclinical data provide promising evidence of the preventative and therapeutic effects of DR in dampening autoimmune and demyelinating responses in experimental models of MS.

Effects of dietary restriction in people with MS

The effects, safety and feasibility of DR in pwMS have been investigated in several studies (Table 2). A 6-month prospective study of 40 pwMS with mild disability (expanded disability status scale-EDSS score ≤ 3) found that Ramadan fasting, lasting approximately 13 hours daily for a month (however fasting periods can vary between 11 to 18 hours depending on year and geographical location), was safe and did not exacerbate disease [56]. Ramadan fasting (14-hour daily fast) in pwMS with mild disability also significantly boosted physical and mental health measures including energy, health perception and emotional well-being [57]. Several interventional DR regimens have been tested in pwMS. A single 7-day cycle of FMD followed by a Mediterranean diet for 6 months improved health-related quality of life metrics and mildly reduced EDSS scores [26]. We compared the effects of 15 days of IF (intake limited to 500 calories every second day) and regular feeding in 16 pwMS undergoing acute MS relapse and receiving corticosteroid treatment [24]. IF was well-tolerated, reduced leptin levels, and recapitulated gut microbiome

Table 2. Summary of studies that tested DR regimens in people with MS (pwMS)

Type of DR and duration	Study design, sample size, subject clinical characteristics	DR effects on inflammatory markers	Main outcomes of DR in pwMS	Reference
Ramadan fasting 13-hour daily fasting period for 1 month	Prospective study (subjects followed for 6 months after Ramadan to assess clinical outcomes) 2 groups – (1) pwMS who fasted during Ramadan, (2) pwMS who did not fast during Ramadan matched for age, gender, EDSS scores and relapse rates (n=40 per group) pwMS with mild disability (EDSS ≤3), type of MS not specified	Not reported	Well tolerated No significant differences in EDSS scores or number of clinical relapses between 2 groups	[56]
Ramadan fasting 14-hour daily fasting period for 1 month	Prospective study (subjects assessed before and after month of Ramadan) 1 group – pwMS who fasted during Ramadan (n=218) pwMS (RRMS) with mild disability (EDSS ≤3)	Not reported	Significantly improves physical health and mental health composites of QOL including role limitations due to emotional problems, emotional wellbeing, energy, health perception, sexual function	[57]
Single cycle of FMD for 7 days (200-350 kcal per day, consisting of vegetable broth/juice and linseed oil) followed by Mediterranean diet (MD) for 6 months	Single-centre, randomised controlled trial 3 dietary intervention groups – (1) 7 days FMD followed by MD for 6 months, (2) ketogenic diet for 6 months, and control diet for 6 months (n=20 per group) pwMS (RRMS) with EDSS ≤6.5	Slight ↓ blood lymphocyte and WBC counts after 6 months of FMD + MD compared to control diet (p=0.07) >20% reduction in lymphocyte count directly after 7 days of FMD in 72% of pwMS given FMD treatment	Well-tolerated (100% compliance rate), safe and feasible Significantly improves health-related QOL measures including overall QOL, change in health, physical health composite, and mental health composite compared to control; mild reduction in EDSS scores	[26]
IF – alternate-day fasting (fasting days restricted to 500 kcal) 15 days	Single-centre randomised controlled pilot trial 2 groups, both given same corticosteroid treatment for acute relapse – (1) pwMS subjected to IF, (2) control group of pwMS eating their regular diet (n=8 per group); no significant differences in age, BMI and EDSS score between groups pwMS (RRMS) experiencing acute clinical relapse at time of study; BMI ≥23	↓ leptin ↓ blood B cell and naive CD4 ⁺ count ↑ Treg cell in vitro suppressive capacity	Well tolerated, safe and feasible, ↓ BMI at day 15	[24]
22% CR or IF (75% calorie reduction for 2 days per week) 8 weeks, with all meals delivered to homes	Single-centre randomised controlled trial 3 groups – (1) pwMS subjected to CR, (2) pwMS subjected to IF, (3) control group pwMS subjected to diet comprising 100% of calorie needs (n=12 per group) pwMS (RRMS) with EDSS <6 and new lesion or relapse within the past 2 years; BMI ≥23	Not reported	Safe and feasible Both 22% CR and IF induce weight loss, improve emotional wellbeing, and have no negative impact on fatigue or sleep quality; ↓ adherence to diet in IF group compared to CR group (measured as per-day difference in calorie consumption from assigned intake)	[58]
22% CR or IF (75% calorie reduction for 2 days per week) 48 weeks (meals delivered in first 8 weeks, followed by self-directed IF (75% calorie reduction for 2 days per week) in last 40 weeks)	Single-centre randomised controlled trial 3 groups in first 8 weeks – (1) pwMS subjected to CR, (2) pwMS subjected to IF, (3) control group pwMS subjected diet comprising 100% of calorie needs (n=12 per group); followed by transition to IF for all groups pwMS (RRMS) with EDSS <6 and new lesion or relapse within the past 2 years; BMI ≥23	Not reported	Safe and feasible, but poor adherence (only 16% of remaining participants reporting adherence to IF at 48 weeks) No significant differences in weight or patient-reported outcomes (fatigue, sleep quality, quality of life)	[59]
22% CR or IF (75% calorie reduction for 2 days per week) 6 months	Single-centre randomised controlled trial Participants allowed to select either (1) CR (n=11) or (2) IF (n=8); 5 CR and 5 IF participants randomised to receive weekly text message communication pwMS (RRMS) receiving natalizumab with BMI ≥25	Not reported	Safe and feasible with 50% adherence Text message communication did not improve adherence or patient-reported outcomes (fatigue, sleep quality, quality of life)	[59]
TRF (16-hour daily fasting period) 6 months	Single-centre randomised controlled trial 2 groups – (1) pwMS undergoing TRF, (2) control group of pwMS eating their regular diet (n=12 per group) pwMS (RRMS) receiving natalizumab	Not reported	Safe and feasible, with 83.3% adherence No significant differences in weight or patient-reported outcomes (fatigue, sleep quality, quality of life)	[59]

Abbreviations: ↑, increase; ↓, decrease; BMI, body mass index; CR, calorie restriction; DR, dietary restriction; EDSS, expanded disability status scale; FMD, fasting mimicking diet; IF, intermittent fasting; pwMS, people with MS; QOL, quality of life; RRMS, relapsing-remitting multiple sclerosis; Treg, regulatory T cell; TRF, time restricted feeding; WBC, white blood cell.

alterations seen in EAE mice subjected to IF [24]. An 8-week randomised controlled study assessed the effects of 22% CR and IF (2 days of 75% reduction in energy intake and 5 days of ad libitum feeding) in 36 pwMS [58]. Both dietary regimens were concluded to be safe and feasible and were associated with significant improvements in emotional health, whilst adherence was greater in the CR regimen [58]. The same research group performed 6-month pragmatic randomised controlled trials of 22% CR, IF (75% calorie reduction for 2 days per week) and TRF (in which consumption of all daily calories was limited to an 8-hour interval), with feasibility and patient adherence as primary outcome measures [59]. Whilst all DR regimens tested proved to be feasible, self-reported adherence was much higher for the TRF diet than both CR and IF regimens over 6 months, which both demonstrated poor long-term adherence [59]. Altogether, a variety of different DR interventions have proven to be safe and feasible in pwMS.

Presently, there are several ongoing clinical trials of DR in pwMS. To investigate whether the anti-inflammatory and gut microbiome-modulating properties of IF observed in preclinical studies are recapitulated in pwMS, we are currently conducting a 12-week randomised controlled study investigating the effects of IF (2 fasting days/week) on peripheral blood immunological parameters, metabolic profiles and gut microbiota composition in pwMS (NCT03539094). Other ongoing trials are focused on determining whether DR can improve clinical outcomes, which is currently inconclusive. An 18-month, 3-armed study is comparing the occurrence of new cerebral lesions as measured by magnetic reson-

ance imaging between 111 pwMS randomly assigned either a ketogenic diet, an IF regimen consisting of TRF (fasting for 14 hours per day) and an additional 1 week of fasting every 6 months, or a control vegetarian-based diet (NCT03508414). Lastly, a study is investigating the effects of 15-20% calorie restriction, with or without abstinence from dairy and gluten products, on MS progression as well as immune cell activity and metabolism (NCT04042415).

Potential risks of DR

Whilst no serious adverse effects have been reported in clinical trials of DR in pwMS, it is important to be aware of the potential risks that may accompany DR and potential contraindications that may render individuals unsuitable for DR. Mild symptoms experienced by pwMS undergoing DR included fatigue and headaches [26,58,59], although these may or may not be directly related to DR. An assessment of adverse events occurring during medically supervised water-only fasting for ≥ 2 days consecutively, with a patient cohort not specific to pwMS, described fatigue, insomnia, nausea, headache, hypertension (which was likely incidental due to pre-existing hypertension), presyncope, dyspepsia, and back pain as effects present in more than 25% of visits (from a total of 768 visits), in order of frequency [60]. Contraindications to DR may include low body weight or BMI, pregnancy, very young or old age, comorbidities such as diabetes, and prescription of specific medications. Further, clinical trials have been conducted only in relapsing-remitting MS (RRMS) patients with mild to moderate disability, thus DR may not be appropriate for pwMS with severe disability.

Conclusion and future perspectives

Since the characterisation of obesity as a risk factor for MS, subsequent investigations into the mechanisms underlying this association have implicated the involvement of cytokines from adipose tissue and dysbiotic microbiota in MS. In particular, DR has emerged as an effective method of counteracting the detrimental effects associated with increased adiposity. Indeed, mounting preclinical evidence suggests that DR exerts neuro-protective effects, which are mediated by the modulation of pro- and anti-inflammatory molecules and alterations to the gut microbiome, among other possible mechanisms. Clinical trials have confirmed the safety and feasibility of various DR regimens and demonstrated DR-induced improvements in quality of life measures in pwMS. There is a need for larger and longer randomised controlled studies to produce strong definitive evidence linking DR with improved clinical outcomes, before any clinical recommendations can be made. Long-term patient adherence has been a barrier to determining whether DR can improve clinical disease outcomes and is an important factor to consider when selecting any particular DR regimen. It's important to recognise that DR alone might not be effective in significantly improving clinical outcomes. However, it can be considered a valuable complementary intervention to commonly used disease modifying treatments and future trials should take into consideration the possibility of an integrated approach. Additionally, the potential risks of DR need to be understood and vulnerable populations of pwMS unsuited to DR regimens need to be identified.

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