A Diet Mimicking Fasting Promotes Regeneration and Reduces Autoimmunity and Multiple Sclerosis Symptoms

Highlights

- FMD reduces pro-inflammatory cytokines and increases corticosterone levels
- FMD suppresses autoimmunity by inducing lymphocyte apoptosis
- FMD promotes regeneration of oligodendrocyte in multiple MS models
- FMD is a safe, feasible, and potentially effective treatment for MS patients

Authors

In Young Choi, Laura Piccio, Patra Childress, ..., Friedemann Paul, Markus Bock, Valter D. Longo

Correspondence

vlongo@usc.edu

In Brief

Choi et al. show that cycles of a fasting mimicking diet (FMD) ameliorate disease severity by suppressing autoimmunity and stimulating remyelination via oligodendrocyte regeneration in multiple sclerosis (MS) mouse models. They also show that a similar FMD is a safe, feasible, and possibly a potentially effective treatment for patients with relapsing-remitting MS.

Choi et al., 2016, Cell Reports 15, 2136–2146

June 7, 2016 © 2016 The Author(s).

http://dx.doi.org/10.1016/j.celrep.2016.05.009
A Diet Mimicking Fasting Promotes Regeneration and Reduces Autoimmunity and Multiple Sclerosis Symptoms

In Young Choi,1,10 Laura Piccio,2,10 Patra Childress,3 Bryan Bollman,2 Arko Ghosh,4 Sebastian Brandhorst,1 Jorge Suarez,1 Andreas Michalsen,3 Anne H. Cross,2 Todd E. Morgan,1 Min Wei,1 Friedemann Paul,6,7 Markus Bock,6,7,11 and Valter D. Longo1,4,8,9,11,*

1Longevity Institute, School of Gerontology, and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, USA
2Department of Neurology and Neurosurgery and Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO 63110, USA
3Global Medicine Program, Keck School of Medicine, University of Southern California, Los Angeles, CA 90089, USA
4Department of Neuroscience, Dana and David Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, CA 90089, USA
5Institute of Social Medicine, Epidemiology and Health Economics, Charité University Medicine Berlin, 10117 Berlin, Germany
6NeuroCure Clinical Research Center and Clinical and Experimental Multiple Sclerosis Research Center, Department of Neurology, Charité University Medicine Berlin, 10117 Berlin, Germany
10Co-first author
7Experimental and Clinical Research Center, a joint cooperation between the Charité Medical Faculty and the Max-Delbrueck Center for Molecular Medicine, 10117 Berlin, Germany
8Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at USC, Keck School of Medicine, University of Southern California, Los Angeles, CA 90089, USA
9FOM, FIRC Institute of Molecular Oncology, 20139 Milan, Italy
11Co-senior author
*Correspondence: vlongo@usc.edu

http://dx.doi.org/10.1016/j.celrep.2016.05.009

SUMMARY

Dietary interventions have not been effective in the treatment of multiple sclerosis (MS). Here, we show that periodic 3-day cycles of a fasting mimicking diet (FMD) are effective in ameliorating demyelination and symptoms in a murine experimental autoimmune encephalomyelitis (EAE) model. The FMD reduced clinical severity in all mice and completely reversed symptoms in 20% of animals. These improvements were associated with increased corticosterone levels and regulatory T (T$_{\text{reg}}$) cell numbers and reduced levels of pro-inflammatory cytokines, T$_{\text{H1}}$ and T$_{\text{H17}}$ cells, and antigen-presenting cells (APCs). Moreover, the FMD promoted oligodendrocyte precursor cell regeneration and remyelination in axons in both EAE and cuprizone MS models, supporting its effects on both suppression of autoimmunity and remyelination. We also report preliminary data suggesting that an FMD or a chronic ketogenic diet are safe, feasible, and potentially effective in the treatment of relapsing-remitting multiple sclerosis (RRMS) patients (NCT01538355).

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disorder characterized by T cell-mediated demyelination and neurodegeneration in the CNS (Friese and Fugger, 2005; Pender and Greer, 2007; Sospedra and Martin, 2005). In experimental autoimmune encephalomyelitis (EAE), an animal model for MS, activated myelin-specific T$_{\text{H1}}$ and T$_{\text{H17}}$ cells cross the blood-brain barrier and migrate into the CNS, where they are activated by local antigen-presenting cells (APCs) and promote inflammation (Dhib-Jalbut, 2007; Fletcher et al., 2010; Goverman, 2009; Hemmer et al., 2002). This inflammatory process leads to oligodendrocyte death, demyelination, and axonal damage, which eventually cause neurologic damage (Lucchinetti et al., 1999; Raine and Wu, 1993). Although oligodendrocyte precursor cells (OPCs) can migrate to the sites of MS lesions, they often fail to differentiate into functional oligodendrocytes (Chang et al., 2002; Wolswijk, 1998). 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 immune system function remains largely unclear, underlining the need for novel therapeutic strategies (Wingerchuk and Carter, 2014). Therefore, effective treatments for MS may require not only the mitigation of autoimmunity but also the stimulation of oligodendrocyte regeneration and restoration of a functional myelin sheath. 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 (Fontana et al., 2010; Guevera-Aguirre et al., 2011; Lee et al., 2010; Longo and Mattson, 2014). Moreover, PF or an FMD reverses the immunosuppression or immunosenescence of either chemotherapy or aging through hematopoietic stem cell-based regeneration (Brandhorst et al., 2015; Cheng et al., 2014). Chronic caloric restriction, a ketogenic diet (KD), and intermittent fasting have been shown to help prevent EAE by reducing inflammation and enhancing neuroprotection when administered prior to disease induction or signs (Esquifino et al., 2007; Kafami et al., 2010; Kim do et al., 2012; Piccio et al., 2008), but dietary interventions have not been reported to be effective as therapies for EAE or MS or to promote myelin regeneration.

Here, we report on the effects of low-calorie and low-protein FMD cycles as a treatment in MS mouse models, and we investigate the mechanisms involved. Furthermore, we report preliminary results on the safety and feasibility of a FMD and a KD in patients with relapsing-remitting multiple sclerosis (RRMS).

**RESULTS**

**FMD Cycles Reduce Disease Severity in the MOG<sub>35-55</sub>-Induced EAE Model**

We examined the effects of 3 cycles of a very-low-calorie and low-protein FMD lasting 3 days every 7 days or a KD continued for 30 days in EAE-induced by active immunization with myelin oligodendrocyte glycoprotein 35–56 (MOG<sub>35-56</sub>) (Figure 1A).
FMD Cycles Reduce Infiltration of Immune Cells in the Spinal Cord

To investigate the capacity of FMD cycles to reduce potential autoimmune T cells, we measured circulating white blood cells (WBCs), lymphocytes, monocytes, and granulocytes in naive, EAE CTRL, EAE FMD, and EAE FMD:RF (measured 4 days after returning to a standard ad lib diet) mice after three cycles of the FMD regimen (Figure 2A). The FMD resulted in a temporary 40%–50% reduction in total WBCs, lymphocytes, monocytes, and granulocytes. Upon returning to the standard ad lib diet (EAE FMD:RF), all complete blood counts (CBCs) returned to either naïve or lower levels than those observed in the EAE CTRL, with the exception of granulocytes, indicating that the FMD cycles cause both WBC death and regeneration (Figure 2A).

To determine whether the reduced active T cell number is due to an increase in the number of regulatory T (T reg) cells, we isolated splenocytes from EAE CTRL and EAE FMD mice at day 3, stained them for CD45rB+ and characterized them by flow cytometry. We observed a significant increase (p < 0.05) in CD11c+ dendritic cells in the EAE FMD cohort compared to the EAE CTRL cohort (3.08% ± 0.70% versus 1.46% ± 0.31%), but we did not observe any changes in the number of F4/80+ macrophage cells in the control or FMD-treated groups (Figures 2E and S2B). To determine the effects of the FMD treatment on T cell infiltration in the spinal cord, we measured T cell activation levels. The number of CD4+ T cells and CD8+ T cells in EAE CTRL and EAE FMD mice was similar (Figures S2C and S2D), but the ratio of splenic naive (CD44low) to activated (CD44high) CD4+ T cells was reduced in the EAE FMD cohort compared to the control group (1.95 versus 3.67; Figure 2F). No difference in CD8+ T cells was observed (Figure S2E). Moreover, the total number of effector (CD44high and CD62Llow) T cells was reduced in the FMD compared to the control group, but the ratio of effector (CD44high and CD62Lhigh) to memory T (CD44high and CD62Llow) cells did not change (Figures S2F–S2H). These results indicate that FMD cycles reduce the number of dendritic cells and increase the relative number of naïve T cells, which may explain the reduced autoimmunity caused by the FMD.

FMD Cycles Induce Autoreactive Lymphocyte Apoptosis and Increase the Number of Naïve Cells

To determine whether FMD cycles also reduce the number of MOG-specific antigen-reactive cells, we used a major histocompatibility complex (MHC) tetramer (MOG35–55/IAd) to identify antigen-reactive cells after an FMD cycle in vivo. The number of CD4+ MOG35–55/IAd+ cells was reduced in the EAE FMD cohort compared to the EAE CTRL cohort (5.75% ± 0.51% versus 3.83% ± 0.66% of lymphocytes; *p < 0.05; Figure 2G). To determine whether the reduced active T cell number is due to an increase in the number of regulatory T (T reg) cells, we isolated lymphocytes from draining lymph nodes and spleens of EAE CTRL or EAE FMD mice and analyzed them for CD4+ CD25+ FoxP3+ T reg cells. The FMD cohort showed a 2-fold increase (p < 0.01) in the number of CD25+ FoxP3+–expressing
FMD Reverses EAE Symptoms by Reducing the Level and Reactivity of Established Autoimmune Cells

To determine how the FMD affects the initiation of EAE, splenocytes were isolated from EAE CTRL and EAE FMD mice, re-activated with MOG35–55 peptide and IL-23 ex vivo, and transferred into naive recipient mice to induce EAE. The mice were then subjected to either a control diet or FMD cycles (Figure 3A). The supernatant from ex vivo splenocyte cultures derived from EAE FMD mice showed no difference in TNF-α levels (110.8 ± 14.9 pg/ml versus 97.1 ± 8.4 pg/ml; Figure 3B) but a major reduction (p < 0.01) in the levels of IFN-γ (342.0 ± 29.8 pg/ml versus 46.6 ± 16.6 pg/ml Figure 3C) and IL-17 (850.5 ± 442.0 pg/ml versus 257.4 ± 36.4 pg/ml; Figure 3D). Interestingly, upon in vitro reactivation, both EAE CTRL and EAE FMD had similar levels of TNF and IL-17 differentiated cells (Figures 3E and 3F). To determine whether the immune cells from EAE CTRL and EAE FMD mice

**Figure 2.** FMD Cycles Decrease the Number of Infiltrating T Cells in the Spinal Cord

(A) Total white blood cell (WBC), lymphocyte, monocyte, and granulocyte counts of naive, EAE-CTRL, EAE-FMD, and EAE-FMD:RF (after 3 days of re-feeding) mice after three cycles of the FMD and a matched time point for EAE-CTRL mice.

(B–D) Spinal cord sections (day 14) and quantification at days 3 and 14 after the first sign of EAE for CD11b+ (B), CD4+ (C), and CD8+ (D) (at least six sections per mouse).

(E) CD11c+ isolated from EAE CTRL or EAE FMD mice on day 3, and quantification of cells from the total isolated splenocyte.

(F) CD4+ gated for CD44low or CD44high cells isolated from EAE CTRL or EAE FMD mice, and quantification of percent splenocytes in CD4+ CD44low (inactive) or CD4+ CD44high (active) cells.

(G) CD3+ lymphocytes gated for CD4 and MOG35–55/IAb from EAE CTRL or EAE FMD mice, and quantification of MOG-specific CD4+ cells.

(H) CD4+ CD25+FoxP3+ isolated from EAE CTRL or EAE FMD mice, and quantification of CD25+ FoxP3+ in CD4+ cells.

(I and J) Intracellular staining for either IFN-γ (I) or IL-17 (J) after gated for CD4+ of the naive, EAE CTRL, EAE FMD, EAE FMD:RF and quantification of cell counts.

(K) Quantification of Annexin V+ apoptotic CD3+ MOG35–55/IAb+ cells.

(L) Quantification of CD4+IFN-γ+ of BrdU+ lymphocytes.

(M–O) Serum TNF-α (M), IFN-γ (N), and IL-17 levels (O) (pg/ml) in naive, EAE CTRL, and EAE FMD mice on day 3 after the first sign of EAE.

(P) Serum corticosterone levels (ng/ml) before immunization, at the time of symptom occurrence, or 3 or 14 days after the initial symptom appeared in the control or FMD group.

n = 4–8 per group; mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001, Student’s t-test, one-way ANOVA, and Bonferroni post test. Scale bar represents 200 μm.
have similar encephalitogenic effects, we transferred splenocytes from either donor group (EAE CTRL or EAE FMD) into naive recipients (A, EAE CTRL donor to control diet recipient; and C, EAE FMD donor to control diet recipient). This resulted in a similar disease incidence rate (Figure 3G) and an equally severe EAE disease severity by day 20 (2.38 ± 0.48 versus 2.70 ± 0.75; Figure 3H), indicating that the FMD did not affect the development and function of reactive immune cells in vivo or ex vivo. However, when FMD treatment was initiated after transfer of control donor splenocytes (B, EAE CTRL donor to naive mice with FMD treatment), recipient mice displayed a delayed disease onset (day 12 versus day 16 post-transfer; Figure 3G) and a major reduction in EAE severity scores compared to control mice (2.38 ± 0.48 versus 0.75 ± 0.87; Figure 3H). Taken together, these results suggest that T cell priming in response to myelin antigen occurred normally in the EAE CTRL and EAE FMD groups, but the FMD can reduce the level of the existing autoimmunity.

**FMD Cycles Stimulate Remyelination by Promoting Oligodendrocyte Regeneration**

To investigate whether the reduced demyelination in FMD mice may also be related to enhanced oligodendrocyte regeneration, we first carried out a quantitative image analysis of NG2+ (an oligodendrocyte progenitor cell [OPC] marker) and GST-π+ (a mature oligodendrocyte marker) in spinal cord sections from control or FMD mice (Figure 4A). We observed no difference in the number of NG2+ OPCs in sections taken from EAE CTRL and EAE FMD mice (Figure S3A). However, at day 14, the number of GST-π+ oligodendrocytes was reduced in the EAE CTRL group, but not in the EAE FMD group (886.7 ± 41.6 versus 1,273 ± 200.3; cells per spinal cord section area; p < 0.01; Figure 4B). To assess whether the normal levels of mature oligodendrocytes in the EAE FMD group were due to enhanced regeneration and/or differentiation, EAE CTRL or EAE FMD mice were injected with BrdU at the time of re-feeding (day 10). We observed a major increase (p < 0.01) in the percentage of cells that are double positive for BrdU+ and GST-π+ in the EAE FMD group compared to the EAE CTRL group (42.9% ± 11.2% versus 83.0% ± 13.2%; p < 0.01), suggesting that the FMD promotes oligodendrocyte differentiation from precursor...
Figure 4. FMD, which Protects the Mouse Spinal Cord from Loss of Oligodendrocytes and Enhances Remyelination, Is Safe and Potentially Effective in the Treatment of MS Patients

(A–C) Spinal cord sections isolated at day 14 and quantification for GST-\(\pi\) (mature oligodendrocyte) and BrdU (A), TUNEL and NG2 (oligodendrocyte precursor cells) (B), and TUNEL and GST-\(\pi\) (C) in naive, EAE-CTRL, or EAE-FMD mice.

(F–H) Sections from the corpus callosum region and quantification of cuprizone treated brains, stained with Luxol Fast Blue of the naive control, end of 5 weeks of cuprizone diet (week 0), cuprizone (5 weeks) plus regular chow (2 weeks), and cuprizone (5 weeks) plus FMD cycle (2 weeks).

(I and J) Section from the corpus callosum region and its quantification of the cuprizone treated brains stained with GST-\(\pi\) of cuprizone (5 weeks) plus regular chow (2 weeks), and cuprizone (5 weeks) plus FMD (2 weeks). Quantification is normalized to percent naive GST-\(\pi\) level.

(K–N) Change in quality of life at 3 months in terms of overall quality of life (K), change in health (L), physical health composite (M), and mental health composite (N). The dotted line represents a threshold that is thought to be clinically important (\(\geq 5\) points). Data represent mean ± standard error of the difference (SED); \(*p < 0.05\), Mann-Whitney U test. An increase of \(\geq 5\) points is considered clinically important.

At least 12 sections per mouse were used for quantification; \(n = 4\); mean ± SEM, \(*p < 0.05\), \(**p < 0.01\), and \(***p < 0.001\), one-way ANOVA and Bonferroni post test.
cells (Figure 4C). To assess the effects of the FMD on either OPCs or mature oligodendrocytes, sections were stained with TUNEL, an apoptotic marker, and GST-\(\pi^+\) or NG2\(^+\) (Figure 4D). We observed a significant increase in the number of TUNEL\(^+\) NG2\(^+\) (11.2 \pm 12.2 versus 1.9 \pm 1.4 cells/section) and TUNEL\(^+\) GST-\(\pi^+\) (18.8 \pm 15.2 versus 2.9 \pm 5.3 cells/section) cells in the control group compared to the FMD group (\(p < 0.05;\) Figures 4E and 4F). Taken together, these results indicate that the FMD not only stimulates regeneration and differentiation of oligodendrocytes but also protects OPCs and mature oligodendrocytes from apoptosis.

To investigate whether the FMD-dependent stimulation of oligodendrocyte differentiation and remyelination can occur independent of the observed effects on T cell number and activity, we used the cuprizone-induced demyelinating mouse model (Ransohoff, 2012; Torkildsen et al., 2008). Addition of 0.2% (w/w) cuprizone to the regular mouse diet for 5–6 weeks results in demyelination in the corpus callosum followed by spontaneous remyelination upon re-feeding with regular chow. After 5 weeks of cuprizone treatment, mice were switched to either the control diet or FMD cycles for 5 weeks, and some were euthanized weekly to assess the degree of myelination by Luxol fast staining and GST-\(\pi^+\) (Figures 4G and 4I). As expected, after 5 weeks of the cuprizone diet, a significant reduction in myelin staining was observed in the corpus callosum compared to the naive controls (Figures 4H and 4J). After two cycles, the FMD-treated group displayed increased myelin staining and an increased number of GST-\(\pi^+\) oligodendrocytes compared to the control diet group (Figures 4H and 4J). However, at later time points, we did not observe differences in spontaneous re-myelination between the control diet and FMD cohorts, as it is well established that cuprizone-dependent myelin damage can be fully reversed after removal of the toxin (Figures S3C and S3D). These results indicate that the FMD promotes OPC-dependent regeneration and accelerates OPC differentiation into oligodendrocytes while enhancing remyelination independently of its modulation of the inflammatory response.

A Randomized Pilot Trial to Test the Effects of a FMD or KD in Relapsing-Remitting MS Patients: Evidence for Safety and Feasibility

A randomized, parallel-group, three-arm pilot trial (NCT01538355) was conducted to assess the safety and feasibility of FMD or KD treatment on health-related quality of life (HRQOL) in RRMS patients. 60 patients were randomly assigned to a control diet (CD; \(n = 20\)), KD for 6 months \((n = 20)\), or a single cycle of a modified human FMD for 7 days \((n = 20)\) followed by a Mediterranean diet for 6 months (Figure S4). Baseline characteristics were balanced among the three groups (Tables S1 and S2). The FMD and KD cohorts displayed clinically meaningful improvements in the HRQOL summary scales at 3 months, which included the overall quality of life (Figure 4K) change in health (Figure 4L), a physical health composite (Figure 4M), and a mental health composite (Figure 4N). Also, similar changes were observed in the total HRQOL scales at different time points (Figure S5). Adverse events (AEs) and serious adverse events (SAEs) were reported for 92% (8%) of CD cohort individuals, 78% (16%) of FMD cohort individuals, and 78% (11%) of KD cohort individuals (Table S5). The most common AE was airway infection, and the most frequent SAE was lower urinary tract infection. No indication of an increase in liver enzymes exceeding the normal range was observed in any of the three treatment groups. Also, the interventions were well tolerated, as evidenced by high compliance rates (CD, 60%; KD, 90%; and FMD, 100%). During the 6-month study period, we observed a total of eight relapses: four in the CD group, one in the KD group, and three in the FMD group. In addition to increased \(\beta\)-hydroxybutyrate levels in plasma, we observed a slight reduction in lymphocytes and WBC counts and detected a mild reduction in expanded disability status scale (EDSS) scores in the FMD and KD groups (measured at baseline, month 3, and month 6; Tables S1 and S6). Thus, there was an inverse association between EDSS and HRQOL scores (Table S7). In MS patients the FMD treatment lead to an over 20% drop in the total lymphocyte count (baseline versus day 8; Table S4) in 72% of the patients (13 of 18 FMD-treated patients). WBC counts returned to the baseline levels after these patients were switched to the Mediterranean diet (month 3). Based on the mouse studies, these results raise the possibility that the FMD alleviated symptoms in MS patients by reducing the number of autoimmune lymphocytes. Overall, our study indicates that the administration of FMD and KD is safe, feasible, and potentially effective, but further studies, including analyses such as magnetic resonance imaging (MRI), blinded clinical assessments, and immune assays, are required to determine efficacy.

DISCUSSION

An FMD administered every week was effective in ameliorating EAE symptoms in all mice and completely reversed disease progression in a portion of animals after the onset of EAE signs. By contrast, the KD had more modest effects and did not reverse EAE progression in mice. FMD cycles appear to be effective in the treatment of EAE in mice by (1) promoting oligodendrocyte precursor-dependent regeneration and (2) reducing the levels of microglia/monocytes and T cells contributing to autoimmunity and encephalomyelitis. Our results support an FMD-mediated anti-inflammatory effect possibly involving the upregulation of AMPK or the downregulation of mTORC1, which sense nutrient availability and dictate cell fate (Laplante and Sabatini, 2012). It was shown that mTORC1 couples immune signals and metabolic programming to establish Treg cell function (Zeng et al., 2013). In fact, treatment with the mTORC1 inhibitor rapamycin or the AMPK activator metformin attenuates EAE symptoms by modulating effector T cells and Treg cells and restricting the infiltration of mononuclear cells into the CNS (Esposito et al., 2010; Nath et al., 2009). Therefore, FMD treatment could interfere with T cell proliferation and differentiation and with recruitment of other immune cells, resulting in a decreased recruitment at lesion sites (Figure 5). Some of these effects of the FMD may be triggered by endogenous glucocorticoid production. Glucocorticoids are used to treat MS relapses, but they are generally administered in short bursts, since they can cause AEs such as osteoporosis and metabolic syndrome (Brusaferri and Candelise, 2000; Ce et al., 2006; Roth et al., 2010; Uttner et al., 2005). The FMD may avoid these adverse effects by promoting additional and coordinated endogenous responses. Importantly, FMD cycles also activated OPCs, resulting in remyelin regeneration, as demonstrated...
by accelerated remyelination rate in the cuprizone model (Figure 5). Notably, because it is the alternation of FMD cycles and re-feeding and not the FMD alone that promotes the regeneration and replacement of autoimmune cells with naive cells, the use of chronic restriction or even a chronic KD may not be effective, or as effective, in the treatment of EAE and MS.

Finally, we report that the administration of the FMD and KD in MS patients was safe and well tolerated and resulted in high compliance. We observed positive effects of FMD cycles or KD treatment in RRMS based on changes in self-reported HRQOL and a mild improvement in EDSS (Table S6). However, the lack of a proper Mediterranean diet control makes it difficult to establish whether FMD cycles alone are sufficient to produce these effects. In addition, MRI analyses and adequately blinded clinical assessments (EDSS and multiple sclerosis functional composite [MSFC]), as well as immune function analyses would greatly enhance the strength of the clinical findings. Because, unlike for the mouse experiments, the FMD was only administered to patients only once, it will be important to test the effects of multiple FMD cycles on MS patients in larger, randomized, and controlled trials.

**EXPERIMENTAL PROCEDURES**

**EAE Model**

C57Bl/6 (10-week-old female) mice were purchased from The Jackson Laboratory and immunized subcutaneously with 200 μg MOG35–55 (GenScript) mixed 1:1 with supplemented complete Freund’s adjuvant followed by 200 ng pertussis toxin (PTX; List Biological Laboratories) intraperitoneally (i.p.) at days 0 and 2. For adoptive transfer, spleens from active immunized mice were isolated and red blood cells (RBCs) were lysed. Spleen cells were cultured in the presence of MOG35–55 (20 μg/ml) with rmIL-23 (20 ng/ml) for 48 hr. Cells were collected and re-suspended in PBS, and 15 million cells were injected intravenously. See Supplemental Experimental Procedures for a detailed description of disease severity scoring. All experiments were performed in accordance with approved Institutional Animal Care and Use Committee (IACUC) protocols of the University of Southern California.

**Mouse Fasting Mimicking Diet**

Mice were fed ad lib with irradiated TD.7912 rodent chow (Harlan Teklad), containing 15.69 kJ/g digestible energy (animal-based protein 3.92 kJ/g, carbohydrate 9.1 kJ/g, and fat 2.67 kJ/g). The experimental FMD is based on a nutritional screen that identified ingredients that allow high nourishment during periods of low calorie consumption. The FMD diet consists of two different components, day 1 diet and day 2–3 diet, that were fed in this order, respectively. See Supplemental Experimental Procedures for a detailed explanation of the FMD. Mice consumed all the supplied food on each day of the FMD regimen and showed no signs of food aversion. After the end of FMD, we supplied TD.7912 chow ad lib for 4 days before starting another FMD cycle. Prior to supplying the FMD, animals were transferred into fresh cages to avoid feeding on residual chow and coprophagy.

**Clinical Trial Design**

This study was a three-armed, parallel-group, single-center, controlled, and randomized clinical pilot trial to assess the effects of dietary interventions on HRQOL in RRMS patients. The permuted-block randomization was generated online at http://randomization.com. An investigator blind
to the randomization plan determined the patients’ randomization number before they underwent the randomization step. This study is registered at http://www.clinicaltrials.gov as NCT01538355. The study was approved by the local ethics committee. All participants gave informed written consent according to the 1964 Declaration of Helsinki. See Supplemental Experimental Procedures for detailed descriptions of the clinical trial and diet compositions.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.05.009.

AUTHOR CONTRIBUTIONS


CONFLICTS OF INTEREST

The University of Southern California has licensed intellectual property to L-Nutra that is under study in this research. As part of this license agreement, the university has the potential to receive royalty payments from L-Nutra. V.D.L. has equity interest in L-Nutra, a company that develops medical food.

ACKNOWLEDGMENTS

We thank Dr. Stephen Hauser for insightful comments, Dr. Pinchas Cohen for assistance with fluorescence microscopy, and Nadine Krueger and Gabi Rahn for technical assistance. L.P. is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society (NMSS, JF 2144A2/1) and is funded by Fondozione Italiana Sclerosis Multipila (FISM, 2014/R15) and the Office of the Assistant Secretary of Defense for Health Affairs, through the Multiple Sclerosis Research Program, under award number W81XWH-14-1-0156. The opinions, interpretations, conclusions, and recommendations express in this article are those of the author and are not necessarily endorsed by the Department of Defense. The mouse study was funded by National Institutes of Health (NIH)/National Institute on Aging (NIA) grant AG034906 (to V.D.L.). The human study was funded by Meylin Projekt e.V. and Familie Ernst Wendt Stiftung Stadt Koeln, which were not involved in any decision-making processes relating the study or its participants. The work of F.P. is supported by Deutsche Forschungsgemeinschaft (DFG Exc 257). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIA or NIH. The University of Southern California has licensed intellectual property to L-Nutra that is under study in this research. As part of this license agreement, the university has the potential to receive royalty payments from L-Nutra. V.D.L. has equity interest in L-Nutra, a company that develops medical food.

Received: May 22, 2015
Revised: February 20, 2016
Accepted: April 26, 2016
Published: May 26, 2016

REFERENCES


