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Principal Investigator: Ellen Mowry, MD, MCR

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**A pragmatic trial of dietary program in people with multiple sclerosis
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JHM IRB - eForm A – Protocol

1. Abstract

- a. **Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.**

Background: The increase in multiple sclerosis (MS) incidence over the last 50 years is likely caused by environmental factors. MS affects at least 400,000 people in the US alone, and its incidence has increased in the past 50 years, likely due to changing environmental risk factors. While vitamin D insufficiency, cigarette smoking, and Epstein-Barr virus play a role, they do not account for the entirety of the increase. Recent research suggests that diet may be a critical factor in MS risk and prognosis; the increasingly common “Western diet”, high in sugar, fat, and salt, may influence MS risk and progression by directly modulating the immune system, altering gut bacteria, and changing metabolism.

Furthermore, co-morbidity related to overweight and obesity may be relevant to MS, as obesity results in chronic low-grade inflammation, may contribute to the initiation of neuroinflammatory processes, and appears to be associated with poorer disease outcomes in people with MS. Dietary modification represents an intriguing potential MS intervention, particularly calorie restriction and fasting, which have been associated with reduced pro-inflammatory cytokines, ceramides, and markers of oxidative stress. Beyond these direct biological effects, optimization of weight may lead to improvements in MS-related symptoms such as fatigue and in overall health-related quality of life. As a result, there is a strong rationale for evaluating the benefits of calorie restriction in MS patients as well as strategies to optimize adherence in a real-world setting. Since there are also some data to suggest that restricting calorie intake to a shortened period within the 24-hour day may also be of benefit, we will also evaluate, in those who don’t meet criteria for the calorie restriction protocol or who refuse to participate in it, whether consolidating typical food intake into a shorter period within each 24-hour day leads to improvements in biomarkers of metabolic impairment or in patient-reported outcomes.

Purpose: The first part of our study will evaluate whether minimal support through weekly automated messaging helps people with MS adhere to a calorie restricted diet. It will also evaluate the effect of weight loss, particularly abdominal obesity, on fatigue and health-related quality of life in patients with MS. The second program will evaluate if shortening the period during the day in which people consume their typical food amounts leads to improvements in patient-reported outcomes or markers of metabolism.

Hypotheses: Intermittent reminders about the importance of adhering to a dietary intervention combined with use of a calorie-tracking smartphone application will improve adherence over use of the application alone. Weight loss, especially decreased abdominal obesity, will improve fatigue and quality of life in patients with MS. Even in people who are not reducing their calorie intake, consuming those calories within a shorter time period within each 24 hours will improve metabolic markers and patient-reported outcomes.

2. Objectives (include all primary and secondary objectives)

Primary Objective. To evaluate if automated messaging support helps people with MS adhere to calorie restriction diet. We predict that intermittent reminders about the importance of adhering to a dietary intervention combined with use of a calorie-tracking smartphone application that is intermittently shared with the study team, will lead to better adherence than use of the application alone in overweight and obese people with MS.

Secondary Objectives. A) To assess the effect of weight loss, particularly reduced abdominal obesity, on fatigue, health-related quality of life, and self-esteem in overweight and obese people with MS. Based on the current literature, we hypothesize that weight loss will improve these aspects of patient well-being.

B) To assess if shortening the time period in which typical calories are consumed during the day improves markers of metabolism, fatigue, or health-related quality of life in people with MS. We hypothesize that consolidating calories consumed into a shorter period of the day leads to improvements in metabolism and patient-reported outcomes.

1. Background

The increase in multiple sclerosis (MS) incidence is likely caused by environmental factors. MS affects at least 400,000 people in the US alone, and its incidence has increased in the past 50 years,¹ likely due to changing environmental risk factors. While vitamin D insufficiency, cigarette smoking, and Epstein-Barr virus play a role,² they do not account for the entirety of the increase, supporting a search for other relevant factors. Since environmental factors are more readily modifiable than are genetic factors, there is a crucial need to identify them so that interventions can be made to reduce the consequences of this disabling disease.

Inflammation and neurodegeneration influence MS phenotype. While most patients have relapsing MS at onset, about 10% have progressive-onset MS, and 50% of those whose disease begins with relapses convert to a progressive phenotype. While relapses are thought to be due to demyelinating autoimmune attacks, neurodegeneration likely causes progressive MS.^{3,4} The link between relapses and progression may be explained by inflammation-induced mitochondrial dysfunction that leads to neurotoxicity via increased reactive oxygen species and calcium.⁵

Diet may be a critical factor in MS risk and prognosis. Somewhat in parallel with the increased MS incidence, dietary intake has changed markedly in recent years, with an increased consumption of the “Western” diet, characterized by a high amount of sugar, fat, and salt. Diet may influence MS in several ways, including directly modulating the immune system, altering gut bacteria, or changing metabolism (including modifying oxidative stress or mitochondrial function). The role of diet is interesting in light of recent data suggesting childhood obesity is also a risk factor for multiple sclerosis as well as an additional report suggesting a genetic overlap between MS and cardiometabolic factors.^{6,7}

Co-morbidity related to overweight/obesity may be relevant to MS. Obesity results in chronic low-grade inflammation and may contribute to the initiation of neuroinflammatory processes in MS, possibly through increases in blood-brain barrier permeability or proinflammatory cytokine

release. In addition, obesity-related disorders are also common in MS patients,⁸ and co-morbidity in people with MS appears to be associated with poorer disease outcomes.^{9,10}

Dietary modification represents an intriguing potential MS intervention. **One aspect of diet that shows promise involves calorie intake.** Calorie restriction or fasting prior to the induction of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, is associated with a less aggressive course.^{11,12} There may be several mechanisms by which this occurs. **Calorie restriction and fasting are associated with reductions in pro-inflammatory cytokines** and other inflammatory markers in EAE mice^{11,12} and in humans who are healthy, obese, or have asthma, similar reductions have been observed.¹³⁻¹⁶ Ceramides are elevated in MS patients and pathogenic in vitro;¹⁷ calorie restriction reduces their levels in humans without MS.¹⁵ Calorie restriction also reduces markers of oxidative stress,¹⁵ and thus may reduce mitochondrial dysfunction. Beyond its direct biological effects, optimization of weight may lead to improvements in MS-related symptoms such as fatigue as well as in health-related quality of life, as overweight MS patients have been shown to have lower self-rated measures of well-being than controls.^{18,19} As such, **there is a strong rationale for evaluating the benefits of calorie restriction in MS.** The timing of calorie intake may also be critical, suggesting a possible rationale for consolidating the time period in which typical calories are consumed.

2. Study Procedures

a. Study design, including the sequence and timing of study procedures

Calorie restriction study:

Overview: We will conduct a pragmatic pilot study in which 80 patients with MS who are undergoing monthly infusions with natalizumab and who are overweight or obese are offered enrollment in this trial. Participants who consent will undergo a basic evaluation of total energy expenditure. At baseline, participants will participate in a short training about intermittent versus continuous calorie restriction and will choose the dietary intervention they'd prefer to use. The LoseIt! Application will be installed on the participants' smartphones, and they will receive training in utilizing the application. Baseline assessments of health-related quality of life (HRQoL), fatigue, self-esteem and physical activity will be conducted. Serum and plasma will be collected for future studies.

Participants will then be randomized to one of two groups: 1) to receiving weekly educational text or email messages about the positive health effects of maintaining a normal body mass index, particularly as it relates to MS, or 2) standard of care (no additional contact). At the routinely-scheduled infusions at months 3 and 6, body mass index, waist circumference, and fatigue/HRQoL/self-esteem will be evaluated. Serum and plasma will be collected for future studies.

Enrollment and Randomization: In order to maximize enrollment within the period of support, people with MS who receive monthly natalizumab infusions and meet other criteria (see #3 inclusion/exclusion criteria) will be offered participation in the study. Potential participants may be notified when receiving their regularly scheduled appointment reminder phone call that a study team member may approach them during their upcoming infusion visit about enrollment in the study. The Johns Hopkins MS Center infusion center currently provides natalizumab infusions for

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140 MS patients per month; it is estimated that two-thirds have a body mass index ≥ 25 kg/m². Participants will be randomized by the study statistician 1:1 to the frequent patient activation arm or standard of care.

Study Procedures/Schedule of Events:

Patients will be consented one month prior to the baseline visit so they can be given adequate notice and can be given a stool sample collection kit to bring it at their next infusion. At the consent visit, baseline energy requirement will be determined (see below) and the patient's waist circumference will be measured. Waist circumference will be measured according to NIH protocol (patient standing with feet shoulder width apart, arms crossed over chest, and abdominal muscles relaxed, with measurement taken at the top of the iliac crest, at the end of normal expiration).

At the baseline visit, the following will be done:

- Vital signs and waist circumference
 - i. If waist circumference varies $\geq 10\%$ from the consent visit, baseline energy requirement will be re-calculated
- Patient-Determined Disease Steps
- Health-related quality of life, (Functional Assessment in MS [FAMS]), fatigue (PROMIS fatigue), Pittsburgh Sleep Quality Index (PSQI), timing of sleep (Morningness-Eveningness Questionnaire [MEQ]), self-esteem (Rosenberg Self-Esteem Scale) and physical activity (International Physical Activity Questionnaire) assessments
- Blood for future research collected (30 ml)
- Coaching about the two diet options
- Installation of LoseIt! application on smartphone
- Determination of baseline energy requirement (*see below*)
- Randomization group assigned (participant blinded)
- Stool sample kit collection

At follow-up visits (months 3 and 6), the following will be done:

- Vital signs and waist circumference
- Patient-Determined Disease Steps (month 6 only)
- Health-related quality of life (Functional Assessment in MS [FAMS]), fatigue (PROMIS fatigue), Pittsburgh Sleep Quality Index (PSQI), timing of sleep (Morningness-Eveningness Questionnaire [MEQ]), self-esteem (Rosenberg Self-Esteem Scale) and physical activity (International Physical Activity Questionnaire) assessments
- Blood for future research (30 ml/visit)
- Stool sample kit distribution (at month 3) and collection (at month 6)
- Collection of LoseIt! application data (calorie counts) since prior visit

Determination of Baseline Energy Requirement and Diet Details: The baseline energy requirement (total energy expenditure) of each participant will be determined prior to randomization. This measurement is defined as the number of calories needed to maintain the current body weight. Total energy expenditure will be assessed using validated equations from University of Arizona Calorie Need Estimates, which include an individual's estimated resting

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energy expenditure (based on current weight, height and age) and physical activity (based on the International Physical Activity Questionnaire short form).

Participants will receive education about two diet approaches: intermittent calorie restriction and continuous calorie restriction. For the intermittent calorie restriction group, a standardized percentage reduction to 25% of the baseline energy requirement will be used two days a week. For the continuous calorie restriction group, the same percentage calorie reduction will be provided, but the restriction will take place over an entire seven-day period (i.e. the group will be provided with food containing 78.6% of their baseline energy requirement (calories) each day. All participants will be encouraged to drink water liberally to stave off hunger. If a participant in one group is unable to follow their chosen diet, rather than losing the subject as a drop-out, the subject will switch to other dietary approach.

Adherence Intervention: All subjects will be asked to document adherence using the LoseIt! application, which tracks calorie intake based on food items and quantity consumed. The application will be installed on the subjects' smartphones at the baseline visit, and they will be instructed how to use it.

Subjects will be randomized 1:1, stratified by week of enrollment, to receive electronic adherence support or to the standard of care (no additional contact). The stratified randomization was chosen because participants often receive their infusions at the same time due to the 28-day dosing schedule, and thus may otherwise discuss their impressions of the study with other participants who happen to be infused at the same time. Those who are in the intervention arm will be sent a text message once a week providing encouraging information about the health benefits of weight loss and, where possible, specifically focusing on how it may benefit their MS. They will be asked to send the previous week's log of calories consumed to the research group (this information is readily available using the LoseIt! application).

Calorie Timing Study

Overview: We will conduct a pragmatic pilot study in which 40 patients with MS who are undergoing monthly infusions with natalizumab and who are either not overweight or obese, or who do meet criteria for the calorie restriction study but refuse to participate, are offered enrollment in this trial. At baseline, participants will be assessed to ensure they know how to take pictures with and save them on their smartphones. Baseline assessments of health-related quality of life (HRQoL), fatigue, self-esteem and physical activity will be conducted.

Participants will then be randomized to one of two groups: 1) to not changing their current dietary patterns at all, or 2) to consuming their total daily calories within a period of 12:00 PM and 8:00 PM. Serum and plasma will be collected for future studies.

Enrollment and Randomization: In order to maximize enrollment within the period of support, people with MS who receive monthly natalizumab infusions and meet other criteria (see #3 inclusion/exclusion criteria) will be offered participation in the study. The Johns Hopkins MS Center infusion center currently provides natalizumab infusions for 140 MS patients per month; it is estimated that one-third have a body mass index < 25 kg/m². Participants will be randomized by the study statistician 1:1 to the frequent patient activation arm or standard of care.

Study Procedures/Schedule of Events:

Patients will be consented one month prior to the baseline visit so they can be given adequate notice and can be given a stool sample collection kit to bring it at their next infusion.

At the baseline visit, the following will be done:

- Vital signs and waist circumference
- Patient-Determined Disease Steps
- Health-related quality of life, (Functional Assessment in MS [FAMS]), fatigue (PROMIS fatigue), Pittsburgh Sleep Quality Index (PSQI), self-esteem (Rosenberg Self-Esteem Scale), and physical activity (International Physical Activity Questionnaire) assessments
- Blood for future research collected
- Evaluation of capacity to take and store photos on phone
- Randomization group assigned (participant blinded)
- Stool sample kit collection

At follow-up visits (months 3 and 6), the following will be done:

- Vital signs and waist circumference
- Patient-Determined Disease Steps (month 6 only)
- Health-related quality of life (Functional Assessment in MS [FAMS]), fatigue (PROMIS fatigue), Pittsburgh Sleep Quality Index (PSQI), and physical activity (International Physical Activity Questionnaire) assessments
- Blood for future research
- Stool sample kit distribution (at month 3) and collection (at month 6)
- Collection of photographic data since prior visit

b. Study duration and number of study visits required of research participants.

The study duration is 6 months from the baseline visit. Follow-up visits will be done at the patients' scheduled infusion appointments at months 3 and 6.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Subjects will be blinded to the study hypothesis that additional electronic support will help improve dietary adherence, which could influence and confound the results. Subjects will be told that the study is evaluating how easily changing calorie amounts or timing can be done in the real world and that, in addition to the baseline education, they may receive periodic contact from the study team via text message. For the calorie restriction study, both groups will receive reminder text messages about the month 3 and 6 study visits, which will help prevent unblinding. Further, the randomization strategy employed (clustered by week of baseline visit) will prevent contamination of patients who may discuss the study while getting infused, since the infusions are scheduled on an every 28-day basis and thus participants would co-mingle in the infusion center and may discuss their experience of the study. For the calorie timing study, participants will be sent text messages in the morning twice a week instructing them to capture

all foods and beverages they consume in the next 24 hours on their smartphone and to save those photos.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

This study does not require any changes to the participants' medical therapies, and they will continue to receive routine care.

e. Justification for inclusion of a placebo or non-treatment group.

The non-treatment group in the calorie restriction study is necessary in order to compare diet adherence with the intervention group. This will help us determine whether additional support without too much additional human effort actually helps overweight and obese people with MS adhere to a dietary intervention. The non-treatment group will still be receiving standard of care. The non-treatment group in the calorie timing study will otherwise receive standard of care therapy, and no know risks are associated with continuing food intake as is currently done.

f. Definition of treatment failure or participant removal criteria.

Patient may be removed from this trial if they become pregnant or develop another condition that the investigator believes may put them at risk from ongoing participation.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

When the study ends, or if a participant's participation in the study ends prematurely, they will no longer receive electronic communication from the research team, but will be able to continue using the LoseIt! application on their smartphone.

3. Inclusion/Exclusion Criteria

Calorie Restriction Study:

Criterion	Rationale
Age 18-75 years; meets 2010 criteria for MS	To ensure broadly generalizable to the MS patient population
Body mass index (BMI) ≥ 25 kg/m ²	To ensure that diet does not lead to too low a BMI; ¹³ restricting BMI to exclusively obese patients (>30 kg/m ²) reduces ability to measure safety in non-obese and generalizability; limiting to only overweight patients (e.g. 25 - 30 kg/m ²) reduces generalizability.
No history of diabetes requiring medication	To avoid unpredictable hypoglycemia
Not pregnant, willing to avoid becoming pregnant during the study, and not breastfeeding	To avoid danger to fetus or threats to milk supply or health
No history of an eating disorder	To prevent relapse of eating disorder
No current use of warfarin	To avoid INR fluctuations

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No history of major surgery within past 3 months	To prevent improper wound healing
Have a smart phone with the capability to download and utilize the LoseIt! application	To log food intake throughout the study duration, so adherence to diet modification may be assessed.

Calorie Timing Study:

Criterion	Rationale
Age 18-75 years; meets 2010 criteria for MS	To ensure broadly generalizable to the MS patient population
Body mass index (BMI) < 25 kg/m ² , or if higher, participant was unwilling to enroll in calorie restriction study	Only want to enroll overweight or obese individuals if they are unwilling to restrict calories, as calorie timing study unlikely to lead to weight loss
No history of diabetes requiring medication	To avoid unpredictable hypoglycemia
Not pregnant, willing to avoid becoming pregnant during the study, and not breastfeeding	To avoid danger to fetus or threats to milk supply or health
No history of an eating disorder	To prevent relapse of eating disorder
No current use of warfarin	To avoid INR fluctuations
No history of major surgery within past 3 months	To prevent improper wound healing
Have a smart phone with the capability of taking and storing photographs	To provides study team with time stamps indicating timing of oral intake.

4. Drugs/ Substances/ Devices

- a. The rationale for choosing the drug and dose or for choosing the device to be used. N/A
- b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed. N/A
- c. Justification and safety information if non-FDA approved drugs without an IND will be administered. N/A

5. Study Statistics

a. Primary outcome variable.

Calorie restriction study: Change in waist circumference

Calorie timing study: Change in metabolism biomarkers

b. Secondary outcome variables.

Calorie restriction study: Change in body mass index, number of pounds lost, percentage adherence to recommended calorie intake, changes in health-related quality of life, changes in fatigue and sleep

Calorie timing study: Adherence to photographing foods, changes in health-related quality of life, fatigue, and sleep

c. Statistical plan including sample size justification and interim data analysis.

Summary statistics (mean \pm standard deviation, median and interquartile range, or number and percentages) will be used to describe the outcomes.

Calorie Restriction:

For the primary objective, the impact of increased patient engagement via the smartphone on weight loss (pounds lost, change in body mass index, and change in waist circumference) as well as on the percentage adherence to the recommended calorie intake (as assessed by LoseIt! logs) will be evaluated in t-tests. If there are baseline imbalances in potentially meaningful covariates despite randomization, multivariate approaches accounting for imbalanced covariates will be employed.

For the secondary objective, the primary analysis will evaluate the association of weight loss (change in body mass index as well as change in waist circumference) with changes in health-related quality of life, sleep and fatigue using multivariate linear regression adjusted for age, sex, race, change in physical activity and baseline disability. Whether group assignment (extra smartphone engagement or standard of care) influences this relationship will also be evaluated.

The rationale for measuring change in waist circumference as the primary outcome is that waist circumference, rather than body mass index, is considered a better measure of the risk of metabolic syndrome.²⁰ However, for comparability with previously-published studies and in order to further evaluate the relationship between waist circumference with body mass index, both measures will be obtained.

Calorie Timing:

Metabolomics will be conducted on samples from the participants to evaluate the impact of restricting the hours in which foods are consumed. Primary analyses will be intent-to treat; secondary analyses will investigate with the actual time of day metrics, as assessed by time stamps of smartphone photos, are predictive of changes in metabolism. Secondary outcomes will be assessed as above.

d. Early stopping rules.

Participants who become pregnant, experience weight loss with BMI < 20 kg/m², or who develop another issue that, in the estimation of the investigator, puts them at risk of ongoing participation, the participant will be asked to cease following the diet but will be followed to the completion of the study.

6. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

- Calorie-restriction diet
 - The risks associated with the diets are not known
 - Previous studies of similar diets in humans have not shown any serious risks.
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- Calorie timing diet
 - Participants may be hungrier in the time in which foods are not being consumed
- Venous blood collection
 - Minimal risk of discomfort, bleeding, bruising or infection at needle entry site
 - Rare risk of vasovagal symptoms or syncope
 - Patients are already getting IV placed for their standard of care treatment, from which blood can be drawn to minimize need for additional venipuncture.

- b. Steps taken to minimize the risks.** To avoid risks associated with phlebotomy, proper safety precautions and standard techniques will be followed.
- c. Plan for reporting unanticipated problems or study deviations.** This is a low-risk study, and no major problems are anticipated. We will use the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 to report and grade all adverse events, whether or not they are related to disease progression or treatment. The relationship between an adverse event and the dietary intervention will be determined by the PI. We will appoint a medical monitor and will report diet-related, unexpected serious adverse events to that person and to the IRB within 2 business days. We will provide the medical monitor a log of adverse events at the time of continuing review submission to the IRB annually. Minor study deviations will also be reported to the monitor and to the IRB annually.
- d. Legal risks such as the risks that would be associated with breach of confidentiality.** Standard procedures will be used to protect patient privacy. Only authorized members of the study team will work on the study. Study files and documents will be kept in password-protected, encrypted computers or in locked offices. There are no known legal ramifications associated with breach of confidentiality, however, given current legislation preventing discrimination related to a health care condition.
- e. Financial risks to the participants.**
There are no known financial risks to participants.

7. Benefits

- a. Description of the probable benefits for the participant and for society.**
For the calorie restriction study, the participants may benefit from the study by losing weight, which could potentially improve levels of fatigue and health-related quality of life. Potential benefits to society include more knowledge about methods to improve adherence to calorie restriction diets. This could benefit both patients with MS as well as society in general. There are no known benefits for the calorie timing participants.

8. Payment and Remuneration

- a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.**
No payment is available.

9. Costs

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a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The study procedures will be paid for by the investigators. The LoseIt! application is free. Thus, there are no costs for participants.

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