

Ketogenic diet and fasting diet as Nutritional Approaches in Multiple Sclerosis (NAMS): protocol of a randomized controlled study

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Study protocol

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Abstract

Background Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system in young adults that may lead to progressive disability. Since pharmacological treatments may have substantial side effects, there is a need for complementary treatment options such as specific dietary approaches. Ketone bodies that are produced during fasting (FD) and ketogenic diets (KD) are an alternative and presumably more efficient energy source for the brain. Studies on mice with experimental autoimmune encephalomyelitis showed beneficial effects of KD and FD on disease progression, disability, cognition and inflammatory markers. However, clinical evidence on these diets is scarce. In the clinical study protocol presented here, we investigate if a KD and a FD are superior to a standard diet (SD) in terms of therapeutic effects and disease progression. **Methods** This study is a single-center, randomized, controlled, parallel-group study. One hundred eleven patients with relapsing-remitting MS with current disease activity and stable immunomodulatory therapy or no disease-modifying therapy will be randomized to one of three dietary interventions over 18 months. Dietary interventions are 1) a KD with a carbohydrate intake of 20-40 g/day, 2) a FD with 7 days of fasting every 6 months and 14 hours intermittent fasting daily in between, and 3) a fat-modified SD as recommended by the German Nutrition Society. Primary outcome measure is the number of new T2-weighted MRI lesions after 18 months. Secondary endpoints are safety, changes in relapse rate, disability progression, fatigue, depression, cognition, quality of life, changes of gut microbiome as well as markers of inflammation, oxidative stress and autophagy. Safety and feasibility will also be assessed. **Discussion** Preclinical data suggest that KD and FD may modulate immunity, reduce disease severity and promote remyelination in the mouse model of MS. However, clinical evidence is lacking. This study is the first clinical study investigating the effects of a KD and FD on disease progression of MS. Trial Registration [ClinicalTrials.gov; NCT03508414](https://clinicaltrials.gov/ct2/show/NCT03508414); Registered 25 April 2018, retrospectively registered; <https://clinicaltrials.gov/ct2/show/NCT03508414>

Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory autoimmune disease of the central nervous system. It leads to neurodegeneration and is one major cause for progressive disability and early retirement in young adults (1-3). According to estimates, more than two million people worldwide are affected (4). Relapsing-remitting MS (RRMS) is the most common form of MS and is characterized by relapses and remission as well as systemic inflammation of the central nervous system and progressive neurodegeneration from earliest disease stages (5, 6).

Currently, there is no cure for MS, but several immunomodulatory therapies are available that may slow disease progression (7). However, all of them may have substantial side effects and patients respond differently due to the complex nature of the disease. Consequently, there is a need for complementary therapies such as specific diets that may reduce MS symptoms, improve patient's quality of life and at best delay disease progression. A recent review

reported that up to 70% of MS patients already used alternative and complementary medicine (8).

It is widely accepted that a combination of genetic susceptibility and environmental factors causes MS (9). That nutrition might be among these factors is supported by the fact that MS is more frequent in Western countries than in less-developed ones (10). The so-called Western diet is high in energy, saturated fats and sugars. Of note, a meal high in fat and carbohydrates led to higher postprandial insulin secretion and inflammation than a meal high in fiber and fruits in healthy lean subjects (11). In line with this, a 12-week ketogenic diet decreased insulin resistance and several serum inflammatory markers in overweight men and women (12).

Insulin resistance seems to be more common in MS patients than in healthy controls and is associated with higher Expanded Disability Status Scale (EDSS) scores (13, 14). Elevated serum levels of the pro-inflammatory cytokine IL17 found in MS patients (14) might contribute to this impaired glucose and insulin metabolism (15).

Cerebral glucose hypometabolism in MS patients (16-18) is thought to reflect mitochondrial dysfunction (19) and might result from neurodegenerative processes which are additionally driven by oxidative stress (20). Several studies demonstrated increased markers of oxidative stress and decreased compensatory antioxidative capacity in MS patients (14, 21-23).

Profound reduction of either overall calorie or carbohydrate intake decreases glucose and insulin levels, thereby driving the body to produce ketone bodies from internal or external fats. These ketone bodies provide an alternative energy source for the brain. They might even be more efficient (per unit oxygen) than glucose (24). Furthermore, ketone bodies seem to stimulate mitochondrial biogenesis and reduce mitochondrial permeability (25, 26) and ketogenic diets decrease expression of pro-oxidative biosynthetic enzymes such as arachidonate 5-lipoxygenase (ALOX5) (27). Details on the proposed mechanisms are summarized in (26). Ketogenic and fasting diets (KD and FD) both drastically reduce carbohydrate intake and it has been suggested that ketone bodies produced during KDs and FDs might be crucial for the regeneration of demyelinated axons (20). Thus, both diets seem to be promising dietary approaches in MS.

Fasting does not only induce ketosis, but might also activate autophagy in brain, liver and muscle (28). Autophagy is a key quality-control pathway in cells that removes impaired cell constituents. It is thought to mediate the anti-aging effects of calorie restriction (29). However, autophagy might also be relevant for ketogenic diets. Current evidence from a rat model suggests that a KD attenuated seizure-induced neuronal injury via autophagy (30).

KDs were established as an alternative treatment in pharmaco-resistant childhood epilepsy in the 1920s (31). The diet was developed to mimic the biochemical effects associated with

fasting (32). KDs have also been shown to improve symptoms in other neurodegenerative diseases such as Alzheimer's and Parkinson's disease (33, 34).

Preclinical evidence for the efficacy of KDs and FDs in MS originates from experimental autoimmune encephalomyelitis (EAE), the established animal model of MS. KD slowed disease progression, improved motor disability and hippocampal atrophy, reversed lesions and suppressed inflammatory cytokines and reactive oxygen species (35). A fasting mimicking diet delayed onset and disease progression, which was accompanied by increased corticosterone levels, autoreactive lymphocyte apoptosis and oligodendrocyte regeneration (36). These findings are supported by other EAE studies showing beneficial effects of intermittent fasting and chronic calorie restriction (37-39).

Both diets differ profoundly from the Western diet which is thought to induce gut dysbiosis, low-grade inflammation and neuroinflammation (40). Recent studies gave indications for gut microbial dysbiosis in MS patients compared to healthy controls (41-43). KD might enhance gut microbial diversity (43). Thus, changes of the gut microbiome could be implicated in the effects of our dietary regimens.

We already obtained clinical evidence for the feasibility and safety of KD and FD in MS patients and showed that they might improve health-related quality of life (36). Another more recent pilot study which tested the safety and tolerability of a modified Atkins diet found decreased body mass index, body fat mass, fatigue and depression scores in 20 RRMS patients. This was, however, an uncontrolled study with a 6 months intervention period, a study design that cannot corroborate effects on disease progression (44).

Here we present the protocol of a randomized, controlled clinical study investigating the effects KD and FD compared to standard diet (SD) in RRMS. The SD is a healthy, predominantly vegetarian diet that follows the recommendations of the German Nutrition Society (DGE). MS patients adhering to a similar diet for 12 months showed improved body mass index, metabolic markers and fatigue but no changes in brain MRI outcomes (45).

Thus, we hypothesize that KD and FD are superior to SD with respect to new lesions on cranial T2-weighted MRI after 18 months compared to baseline. Secondary endpoints are safety aspects of the dietary interventions, brain volume, annualized relapse rate, disability progression, changes in gut microbiome, metabolomics of stool and blood, immunological effects, cognition, fatigue, depression, muscle strength, walking endurance and quality of life.

Methods

Study Design

This is a single-center, randomized, controlled, three-armed, parallel-group study conducted at the NeuroCure Clinical Research Center at Charité - Universitätsmedizin Berlin. Recruitment started in April 2017 (ClinicalTrials.gov identifier: NCT03508414). Patients are recruited from all over Germany. Recruitment strategies include specific study calls on the website of the German Multiple Sclerosis Society and distribution of study flyers in specialized neurological practices as well as information events and lectures for patients. We intend to randomize 111 RRMS patients to one of three dietary interventions over 18 months. Interventions are 1) KD with a carbohydrate intake of 20-40 g/day, 2) FD with 7 days of fasting every 6 months and 14 hours intermittent fasting daily in between, and 3) SD that is predominantly vegetarian and fat-modified as recommended by the German Nutrition Society. The institutional review board of Charité - Universitätsmedizin Berlin approved the study and written informed consent is obtained from all participants prior to study entry (Additional file 1 and 2). The study is conducted in accordance with the Declaration of Helsinki in its currently applicable version, the guidelines of the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) and applicable German laws.

Participants

Patient information and informed consent have been prepared in accordance with the guidelines of the institutional review board of Charité - Universitätsmedizin Berlin. Potential participants receive both forms at least 24 hours before one of the study physicians personally explains all study procedures to them. If he or she is willing to participate and had sufficient time to ask questions, written informed consent is given. Afterwards, inclusion and exclusion criteria are assessed. Main inclusion criteria are a definite diagnosis of RRMS according to the 2010 McDonald criteria (46), a stable immunomodulatory therapy or no disease-modifying therapy for at least 6 months, an EDSS score below 4.5 (47) and disease activity within the last two years before study entry. This is defined as at least one new lesion on brain MRI and/or at least one relapse. Recruitment is done in cohorts of 15-25 patients.

Complete list of inclusion criteria

- Relapsing-remitting MS
- 18-65 years of age
- EDSS < 4.5
- Stable immunomodulatory therapy or no disease-modifying therapy \geq 6 months before study entry

- ≥ 1 relapse or ≥ 1 new T2 lesion or ≥ 1 contrast-enhancing lesion on MRI within the last two years
- Agreement that possible incidental findings will be communicated
- Body mass index between 19 and 45 kg/m²
- Ability to give verbal and written consent
- Health insurance

Complete list of exclusion criteria

- Start or change of immunomodulatory therapy < 6 months before or during the study
- Relapse or cortisone treatment within 30 days before study entry
- Clinically relevant metabolic, progressive or malignant diseases
- Intake of >1g/day omega-3 fatty acid supplements
- Significant cognitive-cooperative impairment
- Insulin-dependent diabetes mellitus (type I)
- Participation in another interventional study
- Weight loss diet or loss of more than 5 kg within 2 months before study entry
- Insufficient mental ability for cooperation
- Eating disorders
- Kidney stones
- Therapy with oral anticoagulants
- Pregnancy and breastfeeding
- Suspected lack of compliance
- Smokers (> 5 cigarettes per day)
- Known alcohol and drug abuse
- Inability to give informed consent or apply to the study protocol
- Contraindications for MRI such as metallic implants, cardiac pacemakers, claustrophobia

Discontinuation criteria are withdrawal of consent, subsequent occurrence of an exclusion criterion (e.g. change of immunomodulatory therapy), lack of compliance and medical reasons for stopping the intervention. Participants meeting a discontinuation criterion are offered to attend the remaining study visits for follow up outside the study protocol.

Randomization

Eligibility of patients will be determined at the screening visit by a trained physician. Afterwards, patients will be stratified according to treatment (MS medication yes or no), gender (male or female), and lesion load (low ≤ 15 T2 lesions or high > 15 T2 lesions) and then randomized to either KD, FD or SD using stratified block randomization with variable block length. This will ensure a homogeneous distribution of interventions over these strata. An external statistician who is not involved in the study carries out the randomization according to predefined randomization lists.

Dietary interventions

Nutritional counseling for all three interventions takes place in small groups within 10 sessions over 18 months (5 sessions month 1-3, 5 sessions month 4-18). The group setting facilitates exchange between patients, management of dietary challenges, monitoring of compliance and recording of adverse events. Between group sessions, patients have the possibility to contact their nutritional counselor via email or telephone at any time.

KD: Nutritional counseling instructs patients to establish ketosis by eating 20 g carbohydrates daily for four weeks. Then, patients increase their carbohydrate intake by 5 g each week until they reach their individual maximum (approx. 40 g) to maintain stable ketosis. All carbohydrates relevant for elevating blood glucose are limited to 40 - 50 g/d. In addition, glycemic index and glycemic load of carbohydrates have to be below 50 and 6, respectively. This ketogenic diet is equivalent to a traditional ketogenic diet, but with a liberalized macronutrient composition of 70-80% fats, 15-20% proteins and 5-10% carbohydrates (compared to a traditional ketogenic diet with 90% fat, 6% proteins and 4% carbohydrates). To control and support compliance, patients receive a hand-held ketone-meter to measure and record blood concentrations of ketone body β -hydroxybutyrate at regular intervals at home. Values should be between 0.5-3.0 mmol/L.

FD: Patients in the FD group fast for 7 days every six months. During this intensive fasting episode, patients only consume vegetable juices and vegetable broth yielding a daily calorie intake between 200 and 350 kcal. Tea and water is *ad libitum* to assure sufficient fluid intake. Two days before and three days after fasting patients eat a low-calorie vegetarian diet for preparation and aftercare. The fasting is initialized by an intestinal clearing with laxatives (e.g. Glauber's Salt by FX-Passage). Outside these intensive fasting episodes, patients intermittently fast for 14 hours daily on seven days of the week in order to maintain fasting

effects. For compliance reasons, participants may take one “cheat day” per week at which they follow the fasting rules less strictly. During intermittent fasting, patients are counseled to eat a diet according to the recommendations of the DGE (equivalent to the SD group). Patients additionally attend group sessions every other day during fasting. A physician and a dietician with expertise on fasting monitor these sessions. During the first intensive fasting episode, these sessions are every other day. In the subsequent fasting episodes, the meetings are less frequent (3-4 meetings).

SD: Patients are counseled to adhere to a non-calorie-restricted diet according to recommendations of the DGE. This diet is predominantly vegetarian with reduced consumption of meat, animal fats, eggs and egg products. Low-fat milk and dairy products are recommended due to their calcium content. Dietary recommendations are designed to modify the omega-6- to omega-3 fatty acid ratio to 5:1.

Adverse events

There are no major risks for patients participating in this study. Minor adverse events of a ketogenic diet and fasting can be headaches, feelings of hunger, fatigue, irritability and dizziness. These side effects are transient and will stop after a few days. Patients will be asked for tolerability of the interventions and any adverse events will be recorded. We do not expect serious adverse events due to our dietary interventions.

Outcome parameters

Primary endpoint is the number of new lesions on cranial T2-weighted MRI after 18 months compared to baseline. Secondary MRI endpoint is brain atrophy determined by percent brain volume change. All MRI scans will be performed in a 3-Tesla MRI scanner (Tim Trio, Siemens, Erlangen, Germany) and will be assessed by an experienced evaluator who is blinded to both clinical data and interventional allocation.

Additional secondary endpoints are annualized relapse rate, neurological-functional disability progression (EDSS, Multiple Sclerosis Functional Composite), cognition (Symbol Digit Modalities Test), fatigue (Fatigue Severity Scale), depression (Beck Depression Inventory II), muscle strength (handgrip dynamometer), walking endurance (six min walk test) and quality of life (MSQoL-54). All endpoints will be assessed at baseline, 9 and 18 months by trained and experienced personnel.

For safety monitoring body weight and composition, dietary intake and vital signs are recorded at regular intervals as well as routine laboratory tests of blood count, kidney and liver

parameters in peripheral venous blood. In addition, we want to investigate metabolic, hormonal and immunological effects of the diets. For this, peripheral blood mononuclear cells are isolated for further analysis. In addition, markers of oxidative stress, especially reaction products of anti-oxidative enzymes, are of interest. Polyamine level and cell metabolites will be measured as secondary markers of nutrient availability and autophagy activation in the cell. Stool samples are collected for 16s rRNA sequencing. For a detailed overview of assessments and endpoints see table 1.

We will inform participants about any conspicuous findings and refer them to their general practitioner for further treatment. After completion of the study, participants have the possibility to be referred to appropriate outpatient nutritional counselling.

Power calculation

Based on the results of other MS studies in our research center in comparable patient cohorts, an average of 3 new T2 lesions after 18 months is expected in the SD group. Sample size was calculated with Wilcoxon (Mann-Whitney) rank sum test and significance level was adjusted (Bonferroni) for the two comparisons (KD vs. SD, FD vs. SD). With a sample size of 33 patients per group and a two-sided significance level of 0.025, there is a power of 80% to detect a probability of 0.72, that an observation from group 1 is smaller than group 2. This corresponds to a standardized effect size of 0.83 and an expected mean difference in the number of new T2 lesions in each of the intervention groups of 0.5 compared to the SD group after 18 months (Query Advisor 7.0). We expect a dropout rate of approximately 10% and therefore plan to enroll 37 patients per group, in total 111 patients.

Data management

Each participant will receive a unique identifier upon study entry. This identifier will be used for all data documentation to assure participant's confidentiality. Data will be gathered in source documents and then transferred into paper case report forms. Later all data will be digitized and stored in a central database. To improve accuracy of data entry, entries will be verified for proper format and expected range as well as double-checked. Overall data quality will be assured by an independent monitoring throughout the study. However, due to the minimal risks of our dietary interventions, a data monitoring committee is deemed unnecessary. Data will be stored for 10 years after study completion and then be deleted. Any modification to the current study protocol will be submitted to the institutional review board, all trial participants, and trial investigators. If participant data or biological samples are

supposed to be used in ancillary studies, additional consent has to be obtained from participants.

Results will be personally explained to all study participants, presented on national and international conferences, published in peer-reviewed journals, and disseminated to neurologists, and the medical laity. We will comply with the official eligibility guidelines for authorship for all publications and do not intend to use professional writers.

Data analysis

The primary endpoint (number of new T2 lesions after 18 months) in the intention-to-treat study population will be compared between treatment groups (KD vs. SD, FD vs. SD) with Mann-Whitney U tests with a two-sided (Bonferroni-adjusted) significance level of 0.025. Secondary endpoints will be analyzed in an explorative manner and descriptives will be given using mean and standard deviation for sufficiently normal distributed metric variables, median with limits of the interquartile range [IQR: 25th and 75th percentile] for skewed metric or ordinal data, as well as absolute and relative frequencies for count data.

We do not plan any interim analyses. Missing values will be replaced using multiple imputation (48). Exploratory data analysis of all secondary endpoints will be conducted at a statistical significance level α of 0.05 without adjustment for multiple comparison

Discussion

To date, this is the first study that investigates long-term efficacy of KD and FD on T2 hyperintense lesion load and clinical measures of disease activity and progression in MS patients. It is also the first study that investigates therapeutic effects of repeated prolonged fasting combined with daily intermittent fasting. There are several reasons for the scarcity of such large-scale, long-term studies. Most prominent reasons are of course the high demand of financial and human resources. Further, patients often prefer one particular intervention and are therefore prone to withdraw consent when randomized to an undesired intervention. Once patients have committed to such a study, it is challenging to convey dietary interventions in a way that sufficient motivation is aroused. Only this will ensure dietary adherence for the whole intervention period.

First, we include patients with any MS medication or no medication. From a methodological point of view, including only patients on the same treatment or untreated patients would have been preferable. However, in light of the variety of available treatment options, we would not be able to recruit a sufficient number of patients with the same medication within an acceptable time span. Thus, we decided to include all patients that have been stable on or off

treatment for at least six months. Stratification only differentiates between treatment and no treatment. Having a stratum for every treatment would have resulted in a sample size unrealistic to recruit and finance. Besides, transferability to clinical routine is only given if the study population, at least in principle, reflects the clinical routine with its diverse application of different treatment options.

Second, a dietary intervention study cannot be blinded completely. Thus, expectations and observer bias cannot be ruled out. However, we try to minimize bias by not communicating any longitudinal data during the study to patients and study personnel in contact with them. Even more important, MRI analysts who evaluate the primary endpoint are blinded for both clinical data and interventional allocation.

Third, we employ MRI scans of both cranium and spinal cord to evaluate disease activity before study entry in order to prevent selection bias. The study MRI, however, only analyses the cranium, thereby neglecting spinal cord lesions. This entails the risk of overlooking disease activity. However, newly developing spinal cord lesions in MS are often associated with clinical symptoms. Thus, they would be recorded as a relapse within regular clinical assessments. Furthermore, cerebral lesions are more frequent and, therefore, constitute a reliable marker for disease activity.

Fourth, controlling and ensuring adherence to dietary interventions is rather challenging. This is especially true in an outpatient setting during such a long study period. Some patients might be disappointed with their interventional allocation and surreptitiously include some features of the desired group into their diet. This can only be avoided by close and frequent contacts between counselor and patient. Patients with suspected or known lack of compliance are excluded from the per-protocol analysis.

One great strength of our study is that patients in the KD group measure their blood ketones at home. Many other studies with low-carbohydrate-high-fat diets did not verify ketosis, which limits the interpretation of results. Although we do not know the actual concentrations of ketone bodies in the brain, blood ketone concentrations have been shown to drive brain's overall metabolic rate (49).

Further strengths are the randomized study design, large sample size, long intervention of 18 months and blinded outcome assessment. Moreover, we focus on both MRI based measures of disease activity and progression and several patient-related endpoints, such as fatigue, depression and quality of life.

Considering epidemiological and preclinical data on the one hand and the lack of clinical data on the other hand, this study has the potential to provide essential data on the efficacy of KD and FD. By analyzing metabolic markers, oxidative stress and the gut microbiome, we hope to

shed some light on the mechanisms that underlie these dietary regimens. A better mechanistic understanding might also be applicable to other neurodegenerative diseases.

In conclusion, KD and FD have the potential for a safe and inexpensive complementary treatment option in MS and our study might close the gap between promising preclinical and the lack of clinical data.

Trial Status

This article is based on the study protocol version 1.3 of 30 July 2018. The NAMS study started 24 May 2017. Recruitment will probably continue until March 2020.

Abbreviations

EDSS: Expanded disability status scale, FD: Fasting diet, ICH-GCP: International conference on harmonization of good clinical practice, KD: Ketogenic diet, MRI: Magnetic resonance imaging, MS: Multiple sclerosis, MSFC: Multiple sclerosis functional composite, NAMS: Nutritional Approaches in Multiple Sclerosis, QoL: Quality of life, SD: Standard diet, SDMT: Symbol digit modalities test

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board of Charité - Universitätsmedizin Berlin and written informed consent was obtained from all study participants before study entry.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no competing interests. The final trial dataset will be available to the investigators. The funders of the study are neither involved in the study design nor the interpretation of data or the publishing of the results.

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Authors' contributions

JBS, MB, AM, FP, AMä designed the study and drafted the study protocol. LSB, LF, DL, AP, DS, MB, CK, NS, AMä developed the interventional concept. LSB, AM, FP and AMä procured funding. SKP did statistical planning, sample size calculation and randomization. LSB and AMä drafted the manuscript. All authors read and approved the final version.

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Table

Table 1. Schedule of study visits with assessments and endpoints

Visit	-1 Screening	0 Baseline	1 Start intervention	2	3	4	5
Month	-4 (max)	-1	0	3	9	15	18
Informed consent	x						
Demographics	x						
Inclusion / exclusion criteria	x	x	x	x	x	x	x
Case history	x						
Medication	x	x	x	x	x	x	x
Vital signs	x			x	x	x	x
Bioelectrical impedance analysis	x				x		x
Anthropometric data	x	x		x	x	x	x
Hand grip strength	x				x		x
Safety lab	x			x	x	x	x
Research lab		x			x		x
Urine sample	x			x	x	x	x
Stool sample		x			x		x
Multiple sclerosis quality of life 54	x				x		x
Questionnaire on activities	x				x		x
Beck depression inventory-II	x				x		x
Fatigue severity scale	x				x		x
Physical examination	x				x		x
Multiple sclerosis functional composite	x				x		x
Symbol digit modalities test	x				x		x
Six-minute walk test	x				x		x
Expanded disability status scale	x				x		x
Relapse query	x	x	x	x	x	x	x
AE / SAE query		x	x	x	x	x	x
MRI		x			x		x
4-day food record		x			x		x
Nutritional counselling			x	*	*	*	*

* There are 10 group sessions for nutritional counselling within 18 months. The FD group has additional meetings during their 7-day-fasts at baseline, after 6 and 12 months

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