The Safety and Feasibility of Mediterranean-Ketogenic Dietary Interventions on Gut Health in Parkinson's Disease: A Protocol for an Open-label, Randomized, Crossover Design Clinical Trial (KIM Trial)

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Study protocol

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Abstract

Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, characterized by a constellation of motor and non-motor symptoms. The etiology of PD is not fully understood, however, the early presence of gastrointestinal symptoms and alterations in the gut microbiome suggest a possible intestinal origin. Another pathophysiological feature of PD is an inefficient utilization of glucose by neuronal cells as the main energy source leading to bioenergetic deficits of the brain. Dietary interventions such as the Mediterranean (MeDi) diet and the ketogenic diet (KD) have shown promise in alleviating the gastrointestinal symptoms and bioenergetics deficits of PD, respectively. Nonetheless, classical KDs may unfavorably alter the gut microbiome, e.g., by decreasing short-chain fatty (SCFA) acid levels. Hence, combining the principles of the MeDi and KD may allow us to harness the potential benefits of both these dietary interventions, while maintaining gut health.

Methods

This study will utilize an open-label, randomized, cross-over design to investigate the safety and feasibility of the Mediterranean-ketogenic diet (MeDi-KD) and MeDi diet supplemented with medium-chain triglycerides (MeDi-MCT) in 50 participants diagnosed with PD. Participants will be randomized to start with either the MeDi-KD or the MeDi-MCT intervention. They will adhere to the respective dietary regimens for 8 weeks followed by an 8-week washout period wherein they will return to their pre-study dietary habits. Following the washout period, the participants will start the other dietary intervention for another 8 weeks. Fecal and blood samples will be collected before and after each intervention to examine the biomarkers associated with gut health. The primary outcome measure of this study will be changes from baseline in fecal SCFA levels, particularly butyrate.

Discussion

The primary objective of this study is to investigate the safety of two Mediterranean-ketogenic interventions with respect to gut microbiome health in patients with PD. This study will provide preliminary evidence and guidance for subsequent large-scale clinical trials investigating multi-pronged dietary interventions to treat PD. If successful, it will de-risk future studies on ketogenic interventions by providing vital information about the safety, tolerability, adherence, and feasibility of the MeDi-KD and MeDi-MCT.

Trial Registration

ClinicalTrials.gov Identifier: NCT05469997

Introduction

Parkinson's disease (PD) is the second most common and the most rapidly growing neurodegenerative disease worldwide (1, 2), and remains without a cure or neuroprotective therapy. Gut-related symptoms are common and often precede the diagnosis, suggesting a possible intestinal origin of PD (3). Numerous studies have demonstrated gut dysbiosis in PD with reduced microbial diversity, increased pro-inflammatory capacity, and decreased short-chain fatty acids (SCFA) production as key characteristics (4–9). For instance, transplantation of fecal matter from human donors with PD compared to healthy controls leads to worsening of PD features and inflammation in an alpha-synuclein overexpressing mouse model (4). Moreover, microbiome studies in PD persistently find increased relative abundance of *Akkermansia muciniphila* (4–16), which likely leads to decomposition of the gut mucin layer (6). An operational framework for the role of gut dysbiosis in PD pathology suggests that reduced production of SCFAs, including butyrate, leads to a reduced energy supply for epithelial cells and in turn, disrupt the integrity of the intestinal barrier (6). The downstream effects of a compromised intestinal barrier include, invasion of pathogens, presumed exposure of the intestinal nervous system to lipopolysaccharides and environmental toxins, gut and systemic inflammation, and aggregation of alpha-synuclein fibrils (6). Given strong evidence for a role of gut dysbiosis and inflammation in PD pathogenesis, interest has been growing in the use of dietary interventions to improve symptoms and slow disease progression.

Emerging evidence suggests that both ketogenic diet (KD) (17–22) and Mediterranean(MeDi) diet (23–29) have beneficial and likely complementary effects in PD. Combining principles from both diets would likely optimize dietary benefits. To the best of our knowledge, no clinical trials have yet been performed into the combined KD and MeDi-style dietary interventions in PD.

MeDi diets are primarily but not exclusively plant-based (23). Their promotion of high fiber content intake promotes the production of SCFA and are associated with improved gut microbiome diversity, reduced oxidative stress, and improved insulin sensitivity (24). Adherence to MeDi in PD is associated with a reduced risk of developing parkinsonism or prodromal PD (25–28) and a higher age of PD onset (29).

KDs are high in fat, adequate in protein and very low in carbohydrates (30). Unlike the MeDi, KD stimulates the synthesis of ketone bodies (KB) that are used as an alternative fuel source to glucose (31), the utilization of which is perturbed in the PD brain (32). Fatty acids are converted to KBs such as beta-hydroxybutyrate (BHB) (30–34). BHB, in turn, is used for mitochondrial ATP generation (31) through bypassing the deficient complex I activity in the PD mitochondria (32). Additionally, BHB also exhibits anti-oxidative (35) and anti-inflammatory properties (36) (review by (37)).

KDs have been successfully used for decades to treat pharmaco-resistant cases of epilepsy (38), and more recently, in diabetes (39), obesity (40), and neurological disorders (21, 22, 41). Small scale pilot trials in PD report improved Movement Disorder Society – Unified Parkinson's Disease Rating Scale (MDS-UPDRS) scores (17), cognitive performance (18) and non-motor symptoms (19) (recent review by (20)).

Another method for inducing serum ketone levels is by consumption of ketogenic medium-chain triglycerides (MCTs; 8-carbon tricaprylin, 10-carbon tricaprin) (42). MCTs are converted to KBs, which can readily cross the blood-brain barrier and the mitochondrial membrane and be utilized as an efficient energy source (42). MCTs can elevate the blood KB levels without the need for carbohydrate intake restrictions or calory deficiency, however, the body will not endogenously produce KBs (i.e., enter the state of ketosis) in the absence of the aforementioned restrictions (42). Therefore, MCT oil supplementation yields a milder elevation in KBs than the KD, however, the ketogenic benefits appear to be largely preserved as shown in studies of epilepsy (43) and cognitive impairment (44, 45). For instance, it has been shown that consumption of MCT supplements can increase brain ketone metabolism by 230% in patients with mild cognitive impairment accompanied by significant cognitive improvements (44, 45).

Studies of classical KDs in other neurological conditions have reported significant alterations within the gut microbiome, including an increase in *Akkermansia* (46). For instance, it has been reported that classical ketogenic diets result in a significant increase of *Akkermansia* in mice models of epilepsy (47). *Akkermansia* plays a critical role in the anti-seizure effect of the KD, which blooms by more than 30% within days of starting the diet (47), however, given the aforementioned increased levels of Akkemansia in PD, a further increase might be detrimental. The classic KD can also reduce fecal SCFA levels, which are important in promoting gut health (48).

By combining the principles of MeDi diet with KD, we hope to leverage the gut-health promoting aspects of the former with bioenergetics benefits of the later in a safe manner.

Methods

Overview and Aims

This is a proof-of concept, open label, randomized, cross-over study investigating the safety and feasibility of MeDi-KD and the MeDi diet supplemented with MCT (MeDi-MCT) in patients with PD (ClinicalTrials.gov Identifier: NCT05469997). Each intervention period will last for 8 weeks and is separated by an 8-week washout period.

The primary aim of this trial is to examine the safety of MeDi-KD and MeDi-MCT interventions with respect to gut health focusing on SCFA production. Specifically, the primary outcome measure with respect to this aim is changes from baseline in fecal butyrate levels as reduced SCFA and butyrate levels are key findings in PD (5–9) and can lead to increased systemic and neuronal inflammation (6). Although the immunomodulatory effects of SCFA's are complex with both pro- and anti-inflammatory functions (4, 49, 50), reduced faecal butyrate levels are best established as detrimental in PD, correlating with age of onset and inversely correlating with motor and non-motor symptoms in PD (5).

Other measures of gut health – such as changes in measures of gut inflammation (using fecal and blood calprotectin as the biomarker), gut-barrier integrity (using fecal and blood zonulin as the biomarker), and

the taxonomic composition of gut microbiome before and after each 8-week intervention (51–53) – will be also assessed as secondary outcome variables.

The secondary aim of this study is to examine feasibility and adherence to the two diets. We will assess retention and adherence rates and perform qualitative participant interviews on the feasibility and barriers to following the dietary interventions. Adherence will be measured with weekly pseudo-random one-day food diaries and weekly blood BHB level self-monitoring.

Lastly, we will explore the effects of the intervention on clinical outcome measures and biomarkers of systemic inflammation. We will perform PD motor and non-motor clinical assessments, including gastrointestinal, cognitive, mood, and behavioral symptoms to compare the outcomes of the two interventions and explore correlations between the clinical measures and adherence to the interventions, KB levels, and inflammatory biomarkers.

Study Design

This safety study will consist of two 8-week interventions (MeDi-MCT and MeDi-KD) in random order separated by 8-weeks washout period (i.e., returning to pre-study dietary habits), using a cross-over design. The 8-week intervention period is based on previous ketogenic interventions that have observed significant changes in the microbiome in trials of 8 weeks duration or less (48, 54, 55). Given that the primary outcome measures of this study – namely changes in gut microbiome function and inflammation – are not expected to be driven by placebo effects, neither intervention will be placebo-controlled in favor of a larger sample size and higher analytic power.

Recruitment Procedures The aim is to recruit 50 participants with a diagnosis of PD (according to MDS criteria (56)) confirmed by their treating neurologist. Participants will primarily be recruited from the Pacific Parkinson Research Centre (PPRC) and the tertiary UBC Movement Disorders Clinic. PPRC sees over 2,500 patients with Parkinson's disease per year, most of whom actively participate in research (57). Recruitment will also be supplemented through the REACH BC platform (58), BC Brain Wellness Program (59), communications of the Djavad Mowafaghian Centre for Brain Health, the Vancouver Coastal Health Research Institute, and social media communications. If individuals from other clinics/centers are interested in participating in this clinical trial, they may reach out to a study team member at the PPRC for more information. A diagnosis of PD must be confirmed by the individual's most recent consult letter from their treating neurologist.

Consent and Screening Willing and interested potential participants will meet with a study team not involved in their clinical care either remotely or in-person at the UBC Movement Disorders Clinic to discuss the study, what participation entails, eligibility criteria, and the risks and benefits of participation in this trial. Potential participants will then be given the opportunity to ask any questions they have and will be given time to decide whether participation is suitable.

Following the obtainment of an informed consent, the participants will complete the screening assessments, including the Montreal cognitive assessment (MoCA), the Beck Depression Inventory-II (BDI-II), the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), as well as a past medical history and medication review. Table 1 outlines all the inclusion and exclusion criteria for this trial. Individuals must meet all eligibility criteria in order to participate in the study. There is no limitation on concomitant medication while taking the MCT supplement, however, we include the use of probiotic in the last 4 weeks and antibiotics in the last 3 months prior to the trial, and immunomodulatory agents as exclusion criteria.

Table 1
Inclusion and exclusion criteria for the trial.

Inclusion Criteria	Exclusion Criteria
Age between 40–85 years	Atypical parkinsonism
PD diagnosis based on Movement Disorder Society (MDS) criteria (56)	Medical or psychiatric conditions that would prevent full participation in the nutrition intervention
Hoehn & Yahr stage between 1 to 3	Pregnancy
On stable dopaminergic medications for at least one month	Significant dysphagia
	Diabetes on insulin
	Anti-coagulation on warfarin
	Inflammatory bowel disease
	Dementia defined by a Montreal Cognitive Assessment (MoCA) score of less than 21
	Inability to fill in electronic questionnaires or understand study instructions
	Use of immunomodulatory agents
	Probiotic use in the last 4 weeks (except for dietary sources such as yoghurt, kefir etc.), or antibiotic use in the last 3 months prior to the trial
	Use of MCT oil or on KD in last 8 weeks prior to the trial
	Allergic to MCT oil, coconut oil, or coconut

Study visits Fig. 1 summarizes all the events, intervention, and assessments and the timepoints at which they will occur during the duration of the study.

[Location of Fig. 1; Figure is temporarily placed at the end of the document, as per the journal's guidelines]

Figure 1. The study SPIRIT figure. Assessments marked as X* will be conducted as a part of the screening procedures, however, they will also be used as baseline measures. The solid and dashed lines marking the study interventions represent the two randomization possibilities.

In short, if individuals continue to meet the eligibility criteria, they will commence the first preintervention visit (visit 1) wherein a study team member will extract and collect de-identified clinical and demographic data including past medical history, disease age of onset, initial PD symptoms, medications, and anthropometric measurements such as height, weight, and waist circumference. Additionally, a series of questionnaires and assessments measuring the participants' motor and non-motor clinical symptoms and their lifestyle and dietary habits will be administered. Table 2 Outlines all the questionnaires that will be administered and their respective purpose.

Table 2
The clinical questionnaires and the associate purpose for which they will be administered.

Questionnaire	Purpose
MDS-UPDRS	PD severity (60)
Fatigue Severity Scale	Measuring Fatigue in patients with PD (61)
Starkstein Apathy Scale	Measuring Apathy in patients with PD (62)
BDI-II	Assessing symptoms of depression (63)
Parkinson Anxiety Scale	Assessing anxiety in patients with PD (64)
Parkinson's Disease Questionnaire-39	Assessing quality of life in PD patients (65)
Physical Activity Scale for Individuals with Physical Disabilities	Assessing the performance of physical activities (66)
Bristol Stool Scale	Assessing stool consistency (67)
Rome III	Constipation and irritable bowel symptoms (68)
Patient Global Impression of Change	Assessing perception of change due to the interventions
World Health Organization quality of life questionnaire	General quality of life
The Canadian version of the Diet History questionnaire	Dietary habits prior to beginning of the study (69)
The National Heath Institute Toolbox Cognitive battery	Assessment of cognitive function (70)

Subsequently, a qualified study team member will collect two tubes of blood (a total of 12 mL) from the participants by venipuncture with an evacuated tube system. Blood collection will occur at the PPRC in the UBC Movement Disorders Clinic. Proper sanitary and disinfection protocols will be followed stringently.

The participants will also be provided with two fecal sampling kits per intervention, each of which will include a sampling scoop, container, instructions, and Bristol stool chart form to fill out. The container will be labelled with the participant's unique study ID. The first fecal sampling kit will be mailed to the participant to be used at home prior to the preintervention visit. The second kit will be used after the participant's first bowel movement following the completion of the 8-week intervention. The samples will be brought back by the participant to the study site during the pre- and post-interventions visits.

Following the completion of the initial assessments and the blood sample collection, the participants will be randomly allocated to start with either the MeDi-KD or the MeDi-MCT with a 1:1 ratio, using variable block sizes. Randomization will be computer-generated (e.g., using the Blockrand package in R) and conducted by an independent statistician prior to the enrollment of the first participant. Subsequently, MCT oil supplies will be provided to those randomized into the MeDi-MCT intervention arm. Relevant instructions on the dosage will be provided by the registered dietitian. Moreover, all participants will be provided with a study journal, which they will use to track their daily intake of the MCT oil supplement (those in the MeDi-MCT arm), bowel movements, pseudorandom one-day summary of their food intake, and any adverse reactions. The procedures described above (except for the randomization and administration of the DHQ questionnaire) will be repeated during the subsequent pre-intervention visit (visit 3) prior to commencing the second intervention phase.

Following the completion of the pre-intervention visit, the participants will spend the next 8 weeks in the respective intervention arm (see Interventions below for more information). Phone check-ins will be completed at the week 4 mark of each intervention phase during which compliance and adverse events will be queried (Participants will be provided with the relevant contact information of the research team and asked to report adverse events at any time during the duration of the study). Additionally, at each check-in, the participants will be asked to remotely complete a number of questionnaires assessing their general quality of life.

Following the completion of the 8-week intervention, the participant will be asked to complete a post-intervention visit (visits 2 and 4). Post-intervention visits will be scheduled within the 8th week of the respective intervention period. The participants will be asked to complete the series of questionnaires, clinical assessments and provide another blood sample. Moreover, they will be asked to bring all study supplement boxes with all used and unused bottles (if they started the study in the MeDi-MCT arm) and the blood ketone monitor to the in-person visit. During these visits, overall compliance to the intervention will be assessed and the feasibility and barriers to following the respective dietary interventions will be queried. Some of the visit procedures again may be completed remotely by accessing our electronic data

capture system, telephone, or videoconferencing, prior to coming to the clinic for a brief in-person portion of the visit.

After completing the first 8-week intervention, the participants will start the 8-week washout period, where they will return to the same lifestyle and dietary habits they were following prior to the start of the study. We will capture baseline dietary habits with the food frequency questionnaire at the first visit. Similar phone check-ins will be conducted at week 4 of the washout period. There will be no in-person visits scheduled for the washout period. Dietary changes lead to gut microbiome changes within days (71), therefore, the 8-week washout period should be sufficient to avoid any carry-over effects.

Lastly, approximately 30 days after the final visit, a phone check-in will be completed with participants to enquire about any changes in their health.

Interventions

MeDi-KD

In the MeDi-KD group, participants will be prescribed a modified MeDi-KD.

In accordance with ketogenic dietary principles, carbohydrates will comprise about 10% of total daily calorie intake while the remaining calories will derive from primarily plant-based fat sources (~ 70–75% of daily caloric intake) and lean proteins (~ 15–20% of daily caloric intake). The ketogenic ratio (the ratio of fat to carbohydrates) will be titrated from 1:1 to 3:1 during the first week. The MeDi component of the diet will encourage the participants to consume more green leafy vegetables, nuts, and olive oil, while limiting the consumption of processed or fried food, red meat, full-fat dairy, and sweets. Participants will be provided with weekly meal plans and recipes curated by a registered dietitian (RD). Furthermore, the RD will continuously monitor the participants' safety while coaching them to ensure continuous adherence to the interventions using motivational counselling techniques.

MeDi-KD is not associated with any significant risks (54, 72). Contrary to the classical KDs that mainly rely on animal-based fats (73), the MeDi-KDs utilize plant-based fats as the primary source of calories. High consumption of animal-based fats can be associated with an increased risk of developing coronary heart disease (21), however ,the plant-based fat sources used in the MeDi-KD have been shown to reduce the risk of hypercholesterolemia and other blood lipid abnormalities (72).

Moreover, classical KDs are associated with alterations within the gut microbiome, including a reduction in the SCFA butyrate (48) and a reduction in the microbial diversity of the gut (74, 75). Although it has not yet been established whether these alterations are harmful or not, the MeDi-KD circumvents these potential risks by allowing for a higher intake of vegetables, particularly those rich in fiber, which are essential for production of SCFAs including butyrate. Previous studies investigating the MeDi-KD in Alzheimer's disease did not observe any changes in the microbial diversity of the gut (54).

We will monitor the participants closely through regular check-ins and symptom diaries, particularly, with respect to gastrointestinal symptoms.

MeDi-MCT

In the MeDi-MCT group, the participants will adhere to the MeDi diet as described above, but with a greater allowance for carbohydrates and natural sweets such as fresh fruits. In addition, they will be asked to take two daily doses of MCT oil. MCT oil is extracted from coconut oil and will provide additional energy for the body (42).

During the first two weeks, participants will be instructed to gradually increase their MCT oil intake until one of the following thresholds are reached:

1. The dose is not tolerable.
2. The limit of the recommended dose on the supplements' label has been reached (i.e., < 40 mL of MCT oil supplement/day).

The MCT oil supplement that will be used in this study is Nutiva MCT oil (Nutiva Inc.). This product is approved by Health Canada (Natural Health Product Number: 80086912) and is thus regarded as safe within the approved condition of use (i.e., source of medium-chain fatty acids which supports energy production in the body at a cellular level [ATP]). Each serving of this product provides 130 Calories from MCTs (14 g) with a C8-C10 ratio of 60:40.

Nutiva MCT oil can be mixed into any beverage of choice, but it cannot be used for cooking. The MCT dose will be gradually titrated from 5 mL (1 teaspoon) to 15 mL (1 tablespoon) twice daily during the first week. If blood ketone levels (see assessment of compliance below for more information) have not reached a minimum of 0.3 mM/L by the end of the first week and if tolerability of the supplement remains positive, the participant will be asked to increase the dose to 20 mL of MCT twice daily.

The most common associated side effect of MCT oil is gastrointestinal (GI) upset, which are typically mild and transient in nature and can be treated with over-the-counter medication and stopping the supplement. The dose titration during the first week should minimize the risk of developing GI symptoms, however, we will monitor the participants for such symptoms throughout the intervention period. Previous studies have determined that 50 g of MCT per day is safe and well tolerated by the participants (76).

Assessment Of Compliance

Dietary adherence will be fostered with a multi-pronged strategy following manuals and standard operating procedures similar to prior large scale, successful dietary interventions (27, 71). All participants will have two individual sessions with an RD in the first two weeks of each intervention, and weekly check-ins for the remainder of each intervention thereafter. To ensure adherence to the protocol and to

successfully address barriers and challenges, individual sessions are standardized but will allow for individualization depending on the needs of the participants.

In case of poor compliance (< 70% adherence to treatment) assessed at week 4, the research coordinator will call the participant to develop strategies for improvement. If the participant is unable to improve compliance to at least 70%, they may be discontinued from the study.

Additionally, participants will also be asked to fill in a 3-day food record at the beginning of the intervention (capturing two weekdays and a weekend day) and pseudo-random one-day records at weekly intervals to ensure adherence and successful implementation of the respective protocol. Moreover, blood ketone body levels will be self-monitored by the participants at home once weekly using the commercially available Freestyle Precision (Abbott) device and Freestyle Precision blood ketone strips. The participants will be instructed insert a test strip into the monitor, use a lancing device (Freestyle Lancing Device II and lancets) to prick their fingers and obtain a small drop of blood, and place the blood drop on the test strip. The measurements will be stored in the device until the participants' post-intervention study visit, where the study team members will extract the data. Previous studies have found blood BHB levels to be more accurate in predicting benefits of ketosis as compared to daily urine ketone monitoring, even if blood analyses are done less frequently (77, 78).

In addition to strategies mentioned above, the participants will be requested to bring all study supplement bottles (used and unused) to the post-intervention visit where overall compliance will be determined.

Data Analysis

Fecal Sampling and Analysis

To collect the samples, the participants will be instructed to scoop a sample of their fecal matter and place the scoop into the provided container. A portion of the sample will be suspended in a buffer and another freshly frozen without buffer. With respect to the latter, the participants will be instructed to place that portion of the sample into their home freezer immediately after collection and transport the sample on ice to our lab where it will be stored at -80 degrees Celsius. The fecal samples will be analyzed for the following:

Microbiome readout

We will perform shotgun metagenomics on the Illumina NextSeq 500 platform using the DNA extracted from fecal samples. Microbiome markers will include relative counts of specific taxa including *Akkermansia*, α- and β-diversity, functional pathways, and pro- and anti-inflammatory propensity.

DNA extraction, library preparation, sequencing, and processing will follow locally established procedures. In short, DNA is extracted with a KingFisher robot using the Qiagen MagAttract PowerSoil DNA KF kit (Formerly MO Bio PowerSoil DNA Kit). Following quality control, libraries are prepared with the Illumina

Nextera library preparation kit (Illumina, San Diego, CA, USA). Pair-end sequencing (150 bp x 2) is done on an Illumina NextSeq 500.

Shotgun metagenomic sequence reads are processed with the Sunbeam pipeline. Processing includes adapter removal, read trimming, low-complexity-reads removal, and host-sequence removals with referencing to the Genome Reference Consortium Human Reference 37. The remaining reads are taxonomically classified with the MiniKraken2_v1 database (79). For functional profiling, high-quality (filtered) reads are aligned against the SEED database via translated homology search and annotated to Subsystems, or functional levels, 1–3 using Super-Focus (80).

Fecal SCFA content

SCFA analysis (including butyrate, acetate, propionate) will be carried out in the freshly frozen faecal samples, using a well-established gas chromatography-mass spectrometry (GC-MS) protocol (81). In brief, 1 mL of ice-cold 10% isobutanol will be added to 100–150 mg of fecal sample to extract SCFAs from fecal samples. The supernatant will then be processed and labeled by isobutyl chloroformate. The labeled SCFA samples will be loaded on an Agilent GC-MS system for SCFA analysis.

Calprotectin and zonulin

A consistent feature of gut microbiome dysbiosis in PD is an increase in intestinal inflammatory markers such as calprotectin and increased intestinal permeability markers such as zonulin (5, 51, 52). Both markers are commonly used for diagnosis and assessment of inflammatory bowel disease and irritable bowel syndrome, which are risk factors for development of PD (52). We will measure these markers in both faecal and blood samples. Zonulin will be measured with a competitive binding enzyme-linked immunosorbent assay (ELISA) (52), while calprotectin will be measured with a two-site sandwich ELISA (53).

Blood Sampling and Analysis

Participants will be instructed to fast overnight (12 hours) before the study visits. During the post-intervention visits, the participants will be served a breakfast meal compliant with their dietary intervention (and if on the MeDi-MCT arm, their morning dose of MCT oil), prior to sample collection. Blood samples will be collected one to two hours after the breakfast meal/MCT intake.

The blood samples will be analyzed for the following:

KBs

In addition to the continuous monitoring of Blood BHB levels throughout the intervention period, we will also measure blood KB levels using the blood samples obtained during the study visits. Blood BHB levels will be determined through a coupled enzyme reaction using the BHB assay kit (Sigma-Aldrich®, USA) (82). In short, 0 to 10 µL of a BHB standard solution will be aliquoted into a 96-well plate and diluted to 50

µL using the BHB assay buffer. Blood serum will be deproteinized prior to addition to the reaction. Results will be determined by measuring absorbance at 450 nm.

Inflammatory biomarkers

Inflammation, both systemic and neuroinflammation are strongly implicated in the pathophysiology of PD and increased inflammation is associated with faster disease progression (83). Dietary patterns such as the typical “Western diet” can induce pro-inflammatory events (84), while others such as the Mediterranean diets are anti-inflammatory in nature (85). In PD, higher baseline blood C-reactive protein (CRP) levels are associated with shorter survival (86) and faster motor progression (87). We will perform the high sensitivity CRP (hsCRP) test to measure the levels of CRP. The blood samples will also be analyzed for 46 blood cytokine and chemokine analytes, using electrochemiluminescence assays from MesoScale Discovery, a high sensitivity assay kit.

Demographic and Clinical Information

As previously mentioned, we will collect detailed clinical and demographic information to maximize insights from this trial. All assessments will be collected in the medication “ON” state. Demographics data will include information regarding the participants’ age, sex, race, ethnicity, education, occupational history, and marital status. Clinical data will include the participants’ anthropometric measurements, blood pressure, past medical history, family history, mode of birth, and a list of medications and supplements currently in use. Additionally, several validated clinical questionnaires, as outlined in Table 2, will be administered to assess the motor and non-motor symptoms, quality of life, and dietary habits of the participants.

Statistical Analysis

We will employ repeated measures analysis of covariance where time (pre- versus post-) will be the repeated factor, and age, sex, PD duration, levodopa-equivalent dose, use of entacapone (15), and order of diet intervention will be covariates. Covariates that do not significantly contribute will be dropped from the model. We will perform separate repeated measures analyses for each diet intervention (pre- and post-MeDi-KD and MeDi-MCT, respectively) and will correct for multiple comparisons. The primary outcome analysis will be based on intention-to-treat. Datasets will also be analyzed by sex on an explorative basis. Functional analysis of altered microbiome profiles will be used to understand the biological mechanisms triggered by the respective dietary interventions. In-depth unsupervised analyses of clinical phenotypes, microbiome features, and inflammatory markers will be used to identify empirical groupings among features, interventions and patients.

Data Storage And Confidentiality

Each participant will be assigned a unique study ID upon entrance into the study. This number will not include any personally identifying information. Only this number will be used on any research-related

information collected from participants, including bio-specimens. Only the PI and REB-approved study team members will have access to the key that links participant study numbers to their identifying information and the interim and final trial dataset. Clinical data collected from patient medical records will be de-identified upon extraction.

Data will be stored on Research Electronic Data Capture System (REDCap) as well as on password protected and encrypted computerized files on personal computers at the Djavad Mowafaghian Centre for Brain Health (DMCBH). Hard copies of participant source documents will be stored in a restricted-access office.

Fecal and blood samples will be stored at -80 C in a restricted-access refrigerator at DMCBH before analysis at the Michael Smith Laboratories at UBC. During analysis, all samples will be stored in restricted-access rooms and freezers. All samples will be destroyed in a confidential manner at the end of the study in their respective locations.

The Principal Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

Adverse Event Reporting

Study physicians and the research staff will monitor the study procedures for this trial for overall safety and scientific relevance on an ongoing basis. Study investigators will evaluate every adverse event for safety and causality. The investigator will report the following types of AEs to the Clinical Research Ethics Board: a) serious AND unanticipated AND possibly, probably or definitely related events; b) anticipated adverse events occurring with a greater magnitude or frequency than expected; and c) other unanticipated problems involving risks to subjects or others. These adverse events or unanticipated problems involving risks to subjects or others will be reported to the CREB within 48 hours of it becoming known to the investigator. Serious adverse events and serious unexpected adverse events will also be reported to the Natural and Non-prescription Health Products Directorate (NNHPD) in an expedited manner.

Discussion

To the best of our knowledge, this will be the first study examining the effects of ketogenic interventions on the gut microbiome in patients with PD. KD have shown promise in ameliorating some of symptoms associated with neurodegenerative disorders, however, their application in PD must be done with care and consideration of the gut microbiome health. By combining the principles of KD and the MeDi, we hope to circumvent the gut-related risks of the KD, while harnessing its bioenergetics benefits.

If successful, this trial will de-risk future studies on ketogenic interventions by providing vital information about the safety, tolerability, adherence, and feasibility of the MeDi-KD and MeDi-MCT. We will determine

if the ketogenic interventions in PD are safe or associated with harmful gut inflammation, reduction of butyrate/SCFA levels, systemic inflammation, gut barrier compromise or dysbiosis – particularly with respect to a potential *Akkermansia* bloom. Moreover, we will understand if the MeDi-KD and the MeDi-MCT can elevate blood ketone bodies to clinically relevant levels, while remaining feasible and safe.

A potential limitation of this study will be adherence to the respective dietary regimens for duration of the intervention period. We have estimated a 20% dropout rate and hope to minimize this through continuous provision of motivational counselling by an RD and constant monitoring of adherence through weekly blood-ketone level measurements. Another limitation of this study is the absence of a control treatment. Nonetheless, given that the primary goal of this study is to establish a safety profile for these interventions, omitting a control treatment in favor of higher analytic power is justifiable.

Furthermore, this study will be the first phase of our laboratory's initiative to investigate the efficacy of ketogenic interventions for patients with PD. By the end, we will weigh the safety, feasibility and exploratory efficacy signals to determine which intervention to pursue in future clinical trials. With this information, we plan to conduct a larger clinical trial focused on clinical efficacy, on the most promising intervention, with the inclusion of comparative control measures and more elaborate data collection (e.g., brain imaging data).

Abbreviations

ANCOVA	Analysis of covariance
AS	Starkstein Apathy Scale
BDI-II	Beck Depression Inventory – II
BHB	Beta-Hydroxybutyrate
C-DHQ	Canadian version of Dietary History Questionnaire – II
CRP	C-Reactive Protein
ELISA	Enzyme-linked immunosorbent assay
FFS	Fatigue Severity Scale
GC-MS	Gas Chromatography Mass Spectroscopy
GCP	Good Clinical Practice
hsCRP	High sensitivity C-Reactive protein
KB	Ketone Bodies
KD	Ketogenic Diets
MCT	Medium-Chain Triglycerides
MDS-UPDRS	Movement Disorder Society – Unified Parkinson's Disease Rating Scale
MeDi	Mediterranean Diet
MeDi-KD	Mediterranean-Ketogenic Diet
MeDi-MCT	Mediterranean diet with MCT oil supplementation
MoCA	Montreal Cognitive Assessment
NIHTB-CB	National Institute of Health Toolbox – Cognitive Battery
PAS	Parkinson Anxiety Scale
PASIPD	The Physical Activity scale for Individuals with Physical Disabilities
PD	Parkinson's Disease
PDQ-39	Parkinson's Disease Questionnaire – 39
PI	Principal Investigator
PPRC	Pacific Parkinson Research Centre
UPDRS	Unified Parkinson's Disease Rating Scale
WOCBP	Women of Childbearing Potential

Declarations

Ethics Approval and Consent to Participate

This study will be conducted in compliance with the protocol that was approved by the University of British Columbia Clinical Research Ethics Board (UBC CREB; Reference number: H21-03747), and according to the standards of Good Clinical Practice and SPIRIT guidelines (See Additional file 1 for more information). Any amendments will be submitted to the UBC Clinical Research Ethics Boards (CREB) for formal approval to conduct the study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form has been reviewed and approved by the CREB. The formal consent of a subject, using the CREB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form will be signed by the subject or their legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

Consent for Publication

This manuscript does not contain any individual person's data in any form and hence consent for publication is not applicable here.

Availability of data and materials

Data sharing is not applicable to this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SK contributed to the conception, literature review and design of the trial, corresponding with CREB, and writing the manuscript; SA-C is the PI and contributed to the conception and design of the trial, and writing the manuscript; KT contributed to trial design and writing the manuscript; MS contributed to the trial design and editing the manuscript; PU and JSTL contributed to writing the manuscript; WT, TC, CW, and BF contributed to trial design; AMR contributed to the background literature review. All authors reviewed and approved the manuscript.

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Additional Files

1. Additional File 1.docx – SPIRIT Checklist – The checklist references the location of various SPIRIT criteria within the text

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Figures

		First Intervention Period		Washout	Second Intervention Period	
	Pre-Enrolment	Pre-intervention 1 (visit 1)	Post-intervention 1 (visit 2)	Washout check-in	Pre-intervention 2 (visit 3)	Post-intervention 2 (visit 4)
Enrollment						
Informed consent	X					
Eligibility screening	X					
Randomization and Allocation		X				
Interventions						
MeDi-KD		←	→		←	→
MeDi-MCT		←	→		←	→
Participant Health Assessment						
Demographic Information (sex, age, year of diagnosis, age of onset, initial symptoms)		X				
Medication overview		X	X	X	X	X
Past Medical History		X				
Weight		X	X		X	X
Height		X				
Waist Circumference		X	X		X	X
Other Participant Information						
Family History/Ancestry		X				
Alcohol Use/Smoking		X				
Occupation		X				
Gynecological History		X				
Education and Socioeconomic Status		X				
Clinical Assessments						
Montreal Cognitive Assessment (MoCA)	X*				X	
Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS)	X*		X		X	X
Questionnaires						
Fatigue Severity Scale (FSS)		X	X		X	X
Parkinson Anxiety Scale (PAS)		X	X		X	X
Starkstein Apathy Scale (AS)		X	X		X	X
Beck Depression Inventory, 2nd Ed. (BDI-II)	X*		X		X	X
Parkinson's Disease Questionnaire – 39 (PDQ-39)		X	X	X	X	X
ROME III Constipation Module (ROME III)		X	X		X	X
The Physical Activity Scale for Individuals with Physical Disabilities (PASIPD)		X	X		X	X
Diet History Questionnaire II (DHQ-II)		X				
Bristol Stool Chart		X	X		X	X
National Health Institute Toolbox Cognitive Battery (NIHTB-CB)		X	X		X	X
Patient Global impression of Change (PGIC)			X			X
WHO Quality of Life Questionnaire		X	X	X	X	X
Sample Collection						
Blood Sample		X	X		X	X
Fecal Sample		X	X		X	X
Qualitative Interview			X			X

Figure 1

SPIRIT Figure

Supplementary Files

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