The attached includes:

Protocol: Acute effects of medium chain triglyceride (MCT) nutritional ketosis on Parkinson's Disease (PD) symptoms and biomarkers (MCT-PD)

- Statistical Plan included within the protocol

NCT04584346

Document Date: 4.19.2021

Abbreviated Title: Feasibility and efficacy of ketogenic diet in Parkinson's Disease
Protocol: *20N0153*
Version Date: 04/19/2021
Title: Acute effects of medium chain triglyceride (MCT) nutritional ketosis on Parkinson's Disease (PD) symptoms and biomarkers (MCT-PD)

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Investigational Agents

Drug Name:	Medium Chain Triglycerides oil
IND Number:	Enforcement discretion
Sponsor:	N.A.
Manufacturer:	Liquigen® (KetoCal)

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Acute effects of medium chain triglyceride (MCT) nutritional ketosis on Parkinson's Disease (PD) symptoms and biomarkers (MCT-PD)

Study Description: While three pilot studies of ketogenic diet (KD) in PD have shown either reduction in motor scores (UPDRS) or improved memory/fluency cognitive testing, there are gaps in knowledge of the time course and mechanisms of reported outcomes. Furthermore, only a standard ketogenic diet was studied while there are variations such as MCT oil supplementation shown to increase keto-induction, and other adaptations may improve tolerability and micronutrient content. It is the goal of this proposed inpatient metabolic study to address the initial question of effect size and time course of ketosis. If suggestive of benefit in PD, this pilot study may lead to a subsequent larger study of long-term feasibility and effects on disease biomarkers and disease progression, which might also compare alternate diets of interest in PD such as Mediterranean diet. Thus, a pilot feasibility study is proposed, targeting retention rate >80% and adherence in the outpatient setting. Recruitment of 32 participants is based upon power analysis of secondary outcome, testing the Timed Up & Go mobility test that has reported validity in fall prediction, additionally plotting continuous and serially repeated direct/indirect ketosis measurements and motor as well as non-motor symptoms / exploratory disease biomarkers. It is hypothesized that, compared to a non-ketogenic, standard American diet (SAD, also referred to interchangeably as usual diet, see Section 2.1 table for description), ketogenic diet supplemented by MCT oil (MCT-KD) will improve mobility tested by Timed Up & Go (TUG), as well as akinesia, tremor, and memory/executive function tasks, and will reduce motor and non-motor fluctuations within the acute period of keto-induction and early ketogenic timepoints due to improved mitochondrial function and neurotransmitter signaling.

Objectives: The primary objective is to test the hypothesis that nutritional ketosis (NK) supplemented by MCT oil in a PD cohort (MCT-KD) is feasible for a duration of three weeks. The secondary objective is to show that NK improves PD symptomatology in cognition (improved attention, recall, and executive function), mobility (TUG), and motor function (bradykinesia, akinesia and tremor) within three weeks.

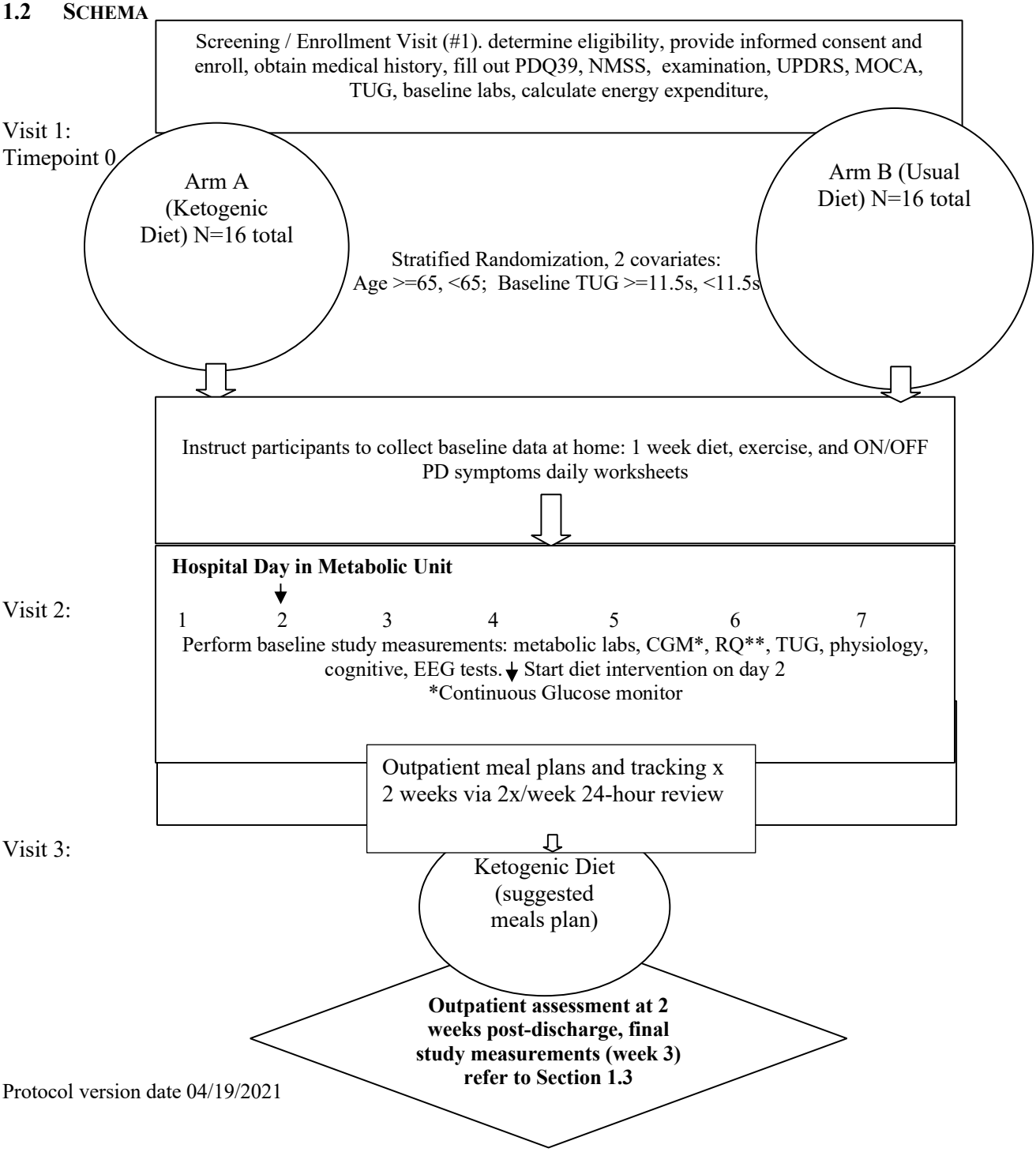
Outcomes: The primary outcome is feasibility, as determined by a composite outcome of recruitment and retention, acceptability and adherence, including full recruitment to study target of 32 participants; completion rate >80% in both inpatient 1-week and outpatient 2-week segments; adherence in the outpatient segment of estimated low carbohydrate diet <10% mean total

	daily energy defined as net carbs); and acceptability defined via exit survey (week 3) 4-point Likert efficacy and likelihood scales on how effective ketogenic diet appears on symptoms and how likely ketogenic diet, with or without MCT oil, participants estimate they will continue the diet on at least an intermittent basis in the future. The secondary outcome is Timed up & go (TUG) mean difference between groups at the end of admission (day 7). Exploratory outcomes include both group comparisons (mean difference) at day 7 and repeated measures days 1-7 between MCT-KD and SAD groups (in most but not all outcomes; refer to section 1.3 Schedule of Activities for details) and at week 3, when both groups combine to a single ketogenic diet group, and within-group pre vs. post intervention comparisons at 1 & 3 weeks for both groups separately (SAD at 0 & 2 weeks of ketogenic diet vs. MCT-KD group at 1 & 3 weeks, respectively) and all participants, in the following: Stroop word-color interference latency (executive function), n-back recall task (attention and working memory), finger tap speed and rhythm (MN and PQ keyboard tasks), accelerometry amplitude for tremor analysis; 9-hole pegboard test (9-HPT) (daily), heart rate variability HF power, total power (resting, daily), cardiovagal testing via deep breathing and Valsalva Maneuver (pre vs. post), mean subjective on vs. off time (pre vs. post), non-motor severity score (NMSS) pre vs. post, keto-induction symptoms, UPDRS (pre vs. post), EEG spectral power by frequency and connectivity analysis (day 1 vs. day 7 vs. week 3), Geriatric Depression Scale (GDS, pre vs. post)
Study Population:	Idiopathic Parkinson's Disease of moderate severity, Hoehn & Yahr Stage 2-4, age >50, usual (carbohydrate predominant) diet at baseline. A total of 32 PD patients will be recruited predominantly from the Parkinson's Clinic at NIH and local sites given necessity of three visits to facilitate randomization and baseline data acquisition. Recruitment will continue as per power analysis for Timed Up & Go (TUG).
Phase:	1
Description of Sites/Facilities Enrolling Participants:	Enrollment will occur at Screening Visit, NIH Clinical Center Outpatient Clinic 5 (OP5). Admission will occur at NIH Clinical Center Metabolic Unit, 7SW.
Description of Study Intervention:	The study intervention is randomization to MCT-KD (ketogenic diet with 80% energy from lipids including Liquigen® (KetoCal) comprising 25% of the daily lipid energy divided twice daily or typical, standard American diet (SAD) x 6 days, followed by 2 weeks ketogenic diet in both groups at home. The proposed diets are isocaloric and have the same ratio of protein (10-15% daily energy) with similar mealtime boluses to limit differences in L-DOPA absorption. Daily activity and exercise plans will be individualized upon admission including use of stationary bike, aiming to maintain baseline levels. Diet with MCT oil will begin on hospital day 2

and be provided to both groups at outpatient day 1 Both diets will be double-blinded (combination of shakes and similar appearing snacks). MCT-KD will feature reduced carbohydrate intake of 5-10% daily energy requirement calculated in the Metabolic Unit. Safety monitoring will be performed by IMM.

Study Duration: 9 months
Participant Duration: 3 weeks

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	**Screening/ enrollment ***	**Day 1 (pre-intervention)**	**Day 2 (diet started)**	**D 3**	**D 4**	**D 5**	**D 6**	**D 7**	**W 3 (post)**
Fill out intake form and determine eligibility	X								
Pregnancy test**	X								
Informed consent	X								
Randomization to Ketogenic Diet or Usual Diet group, stratified 2-factor covariate	X								
anthropomorphic measures (height, weight, abdominal circumference)	X							X	X
Vital signs, including orthostatic (exploratory outcome)	X	X	X	X	X	X	X	X	X
History and Physical Exam	X	X							
Focused Neurologic Exam	X	X							X
Bioelectric Impedance (BIA)	X								X
MOCA	X								
Screening BMP (to establish eGFR), hepatic panel, lipid panel, A1C, beta-HCG if female < age 60	X								

Procedures	**Screening**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 / Post**	**W 3 (post)**
UPDRS part 3	X	X	X	X	X	X	X	X	X
Total UPDRS	X								X
Hoehn & Yahr Stage	X								
Electrocardiogram (ECG)	X*	*(If ECG service is unable during screening, may be performed on Day 1 prior to initation of diet)							
metabolic labs (A1c, insulin, CRP, apolipoprotein panel, HOMA-IR, cortisol, TSH, T3, free T4), BDNF (exploratory outcome), lipid panel (day 7, week 3), A1c (week 3 only)		X						X	X
Non Motor Severity Scale (NMSS) (exploratory outcome)	X								X
PDQ-39 questionnaire (exploratory outcome)	X								X
Resting heart rate variability (2-10 min)		X	X	X	X	X	X	X	X

(exploratory outcome)									
Procedures	**Screening**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 / Post**	**W 3 (post)**
Cardiovagal testing – Valsalva maneuver, deep breathing (exploratory outcome)		X						X	X
Timed up and Go (TUG) AM fasting (secondary outcome)	X	X	X	X	X	X	X	X	X
Simple reaction time (SRT) daily (exploratory outcome)		X	X	X	X	X	X	X	X
9-hole pegboard test (9-HPT) daily (exploratory outcome)		X	X	X	X	X	X	X	X
daily journal of ON/OFF and keto-induction symptoms, (Likert scales) and mood (exploratory outcome)		X	X	X	X	X	X	X	X
EEG spectral analysis and connectivity analysis (phase lag index) days 1, 7 (exploratory outcome)		X						X	X
Respiratory Metabolic Cart quotient (pCO2, :pO2) for restingand energy expenditure	X								X
BMP, magnesium		X		X	X	X	X	***	

POG glc checks		X	X	X	X	X	X	X	
Beta-OHB level twice daily, except once on day 7****		X	X	X	X	X	X	X	X****
Procedures	**Screening**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7 / Post**	**W 3 (post)**
Plasma dopamine twice daily		X	X	X					
Continuous glucose monitoring (Precision Libre Pro)		X	X	X	X	X	X	X	
n-back working memory test daily (exploratory outcome)		X	X	X	X	X	X	X	X
Stroop word color interference test daily (exploratory outcome)		X	X	X	X	X	X	X	X
MN and PQ keyboard tasks		X	X	X	X	X	X	X	X
Safety monitor (telephone interview) survey 2-4 weeks post-study completion									X
Depression screening tool: Geriatric Depression Scale (GDS)	X							X	X
Gastrointestinal motility / symptoms diary (daily days 1-7)		X	X	X	X	X	X	X	

Gastrointestinal Symptom Rating Scale	X	X						X	X
Exit Survey acceptability of ketogenic diet									X
Tracking diet via unscheduled calls*****	X								X
*No Day 0 (7 days of baseline diet and history, then Day 1 is first day of admission) **women of childbearing potential ***repeat BMP and magnesium on days 6 and 7 if abnormal in prior days ****check beta-OHB in fasted state at week 3 visit ***** To occur during week between screening visit/enrollment and day 1 of admission and during 2 weeks after day 7. See Section 4.1 for details.									

2 INTRODUCTION

2.1 STUDY RATIONALE

Three pilot studies of evaluating use of the traditional ketogenic diet in PD have been reported. They showed either reduction in UPDRS, particularly non motor greater than motor scores (Van Itallie 2005, Phillips 2018), or improved cognition (Krikorian 2019). These studies all had limitations, in part related to pilot outpatient design, with gaps or deficiencies identified in the following: monitoring for adherence and reliability of the intervention; degree of ketosis attained; frequency of testing for ketosis; frequency of outcome assessment to obtain adequate signal : noise ratio with respect to biological fluctuations in the disease; reliability of UPDRS rating scale used as primary outcome for small cohort groups; lack of quantitative testing of cognitive and motor functions in the same cohort for comparison; lack of testing for disease mechanism engaged that might account for the proposed benefits.

VanItallie et al reported results of an unblinded, at-home ketogenic diet for 28 days in terms of pre vs. post UPDRS scores of 5 individuals with PD (from 7 with 2 dropouts), 4/5 with Hoehn & Yahr stage 3, who had varying degrees of ketosis mostly high therapeutic range of beta-OHB levels reported (4.8 – 8.9 mM) and one outlier range 1.13 – 1.56 mM). All five participants had decline in total UPDRS score, mostly (4/5 participants) being 21-46% from baseline. There were clear study limitations of placebo effect in study lacking a control group; lack of rater blinding and placebo control; lack of reliable ketosis monitoring aside from infrequent (unreported frequency) serum betaOHB, whereas daily ketone measurement was only urine dipstick and therefore qualitative; lack of monitoring of the diet relying primarily upon self-report via telephone and intermittent patient visits to the dietician; limited study size, and strict 4:1 standard ketogenic diet regimen with 2 drop outs of 7 participants and additional 2/5 remaining participants with reported dietary relapse.

Phillips et al addressed some of these issues by comparing two randomized groups, a comparatively less stringent ketogenic diet vs. a low-fat diet prescribed by meal plans, each with approximately 18% protein and either 79% lipid / 3.7% net carb (KD) or 23% lipid / 59% net carb (low-fat diet), the latter also with greater dietary fiber, stratified by estimated daily energy expenditure, for longer duration (8 weeks) and checking for ketosis monitoring by daily (AM fasting) beta-OHB levels by a validated fingerstick ketone meter as well as monitoring adherence through daily ticking of meal plans that were outlined for the entire study period. However, this study also had some limitations including infrequent data capture and diet monitoring relying upon self-report, precluding correlative analysis between ketosis value and clinical rating scale (UPDRS) performed only pre vs. post intervention. Another possible limitation was mean beta OHB level being only 1.19 in the KD group, if greater degree of ketosis might be beneficial.

Krikorian et al evaluated another less stringent ketogenic diet in a PD cohort with mild cognitive impairment (unlimited protein, <20g carb target), recognizing that impaired glucose tolerance / metabolic syndrome is a risk factor for higher comorbidity in PD than in the general population, and that the literature supports ketogenic diet as a therapy specifically for metabolic syndrome (Hyde et al, 2019). They similarly used pre vs. post intervention data capture of 8 weeks duration, assessing cognition as well as UPDRS, and the dietary manipulation of only carbohydrate may provide a more feasible approach with lower level of change required for participants, however even the less prescriptive guidelines were not exactly adhered to. They followed 10 subjects in KD group vs. 7 in low-fat group, however they reported low level ketosis (mean beta-OHB 0.31 mM by study end week 8) in the low carbohydrate group and mean carb daily intake of 36g (above 20g) in that group. Dietary monitoring was done by patients filling out three-day diaries in each study week. Interestingly, despite the low ketosis values they nonetheless found improvement in short-term memory and verbal fluency in the KD group compared to the high

carbohydrate diet despite no motor differences on UPDRS-III or finger tapping speed, which raises the question of does ketosis matter for symptom improvement (whether cognitive or motor) in PD, as opposed to effects of lipid, carbohydrate or protein metabolism, such as improved insulin sensitivity / insulin brain signaling and increased BDNF expression, benefits attributed to ketogenic diet (please refer to Section 3.2.1 for details) but not specific to it. The question of macronutrient ratio contribution to the cognitive benefit in the study was not tested, and such comparison was not available given that all three macronutrient groups varied proportionally between the two groups. For instance, mean protein intake, which was not controlled, was higher in the low carbohydrate group (123 g vs. 89 g in the high carbohydrate group). Lack of controlling for protein intake appears to be a confounding issue for Parkinson's studies given the results confirmed in multiple studies of levodopa absorption being affected by food intake, for instance large neutral amino acid L-leucine decreased intestinal absorption of levodopa by ~50% in healthy subjects (Lennernäs et al, 1993), which is meaningful particularly in moderate to advanced PD where dopamine-responsive fluctuations occur coupled with the pharmacokinetics of levodopa concentrations, and a single load of 0.4 g protein / kg body weight diminished response of levodopa in PD patients with motor fluctuations (Mena and Cotzias 1975; Nutt et al 1984; Juncus et al 1987; Pincus and Barry 1987).

These limitations form the primary impetus behind the current study. The inpatient design is well suited for a pilot feasibility study, as it offers advantages in providing optimal adherence monitoring and generation of a time series through frequent ketosis values compared to symptom monitoring including repeated clinical, physiology, and cognitive tests that would be more difficult or simply not feasible in the outpatient setting. The overall goal is to test metabolic measures of a standard ketogenic diet with MCT oil supplement in an effort to improve upon feasibility issues identified in the other studies. A corollary objective is to test the effects of ketosis on symptom measures by comparing MCT-KD to a standard, American diet. The following table outlines macronutrient composition of diets proposed for study (italicized) and other diets with available data on ketogenesis or use in PD. While the proposed KD matches others such as Phillips et al in proportion of energy supply from lipids of around 80%, the addition of MCT supplement to replace a portion of the 80% lipids may be a useful adaptation, similar to a study in healthy volunteers that showed more rapid keto-induction and less keto-induction symptoms (Harvey et al, 2019) in order to better and more expeditiously achieve the primary objective of ketosis feasibility.

Table 1 – macronutrient ratios of different diets used in clinical research for PD / other neurologic disorders

	Modified MD-KD (MMKD)	MCT diet	KD*	SAD (high carb diet)	MD	LFD, aka AHAD
Lipid	60-70%	45-70%	80%	30-40%	45-50%	15-20% (<7% saturated, <300mg cholesterol daily)
Protein	25-30%	10-20%	10-15%	15%	15-20%	20-30%

Net Carb	5-10%, max 30 g	10-35%	5-10%	45-55%	30-40%	55-65%
Comment	Studied 12 weeks in obesity (Perez-Guisado et al 2008); in MCI/CN/SMC (Neth et al 2020)	Improved compliance in pediatric epilepsy (Liu et al 2008)	Studied in PD VanItallie et al 2005; Phillips et al 2018; in obesity/MetSx (Hyde et al, 2019); 8 weeks in PD-MCI Krikorian et al 2019	As defined by Stephen Phinney; studied by Krikorian et al, 2019	Widely considered to be rich in vitamins/mineral, less risk of deficiencies	Studied as control group in Phillips et al 2018; Neth et al 2020). AHA Guidelines: rich in fruit/vegetable, whole grains, fish 2+ meals/ week, minimize added sugars,
Ketosis values	Mean 0.31 mM (Krikorian MCI cohort) 0.7 mM (Neth et al – 1.0 mM in SMC vs 0.4 mM in MCI)	Mean increase beta-OHB by 0.7 mM by study day 8-9 of 20 randomized 30mL TID MCT vs. LCT, sunflower oil (Harvey et al, 2019)	Mean AM fasting betaOHB 1.15 mM vs. 0.16 mM in LFD (Phillips et al, 2018)	0 to 0.2 mM (daily fasted state)	No data found	0 to 0.2 mM (daily fasted state)
Glucose / other ancillary data	Glucose decrease by 16 mg/dL (109 to 93) Perez-Guisado 2008; decrease TG (-23 mg/dL) and VLDL (-4.6 mg/dL) increase LDL 17 mg/DL; body weight loss greater than AHAD (Neth et al)		In cohort with MetSx x 4 wks: Reduction of glucose -10 mg/dL, TG -60 mg/dL, dense LDL, plasma total saturated fat, increased HDL (+4 mg/dL), arachidonate compared to medium-carb and high carb diets (Hyde et al, 2019) Increased HDL, LDL, no difference TG compared to LFD (Phillips et al, 2018). HbA1c trend not statistically significant.		Reduced risk of prodromal PD in a populational cohort in Greece (Maraki 2019); consistently reduced LDL, central obesity and Met Sx (Babio et al 2014 PREDIMED trial); many studies with reduced CV risk reduced mortality (Bo et al 2016)	In 20 HV: No change in LDL, HDL, TG in 4 mo. crossover study compared to decline in LDL & TG and increase in HDL after 4 mo. Paleolithic diet (40% lipid, 23% carb, 37% protein -- no dairy, grains, legumes, daily: <1/2 cup potato, <1oz dried fruit, <4oz wine daily, unlimited eggs) (Pastore et al, 2015)
Keto-induction symptoms / side effects	Attrition rate 22.5% (Perez-Guisado), 10% Neth et al and Nagpal et al for MMKD vs. 5% for	Shortened keto-induction (0.5 mM) from day 5 to day 3, vs LCT (sunflower) oil; decrease overall keto-induction symptoms but	Attrition rate 28% VanItallie et al, 18% Phillips et al (9% attributed to diet). No major adverse event. Initial symptoms of increased			

	AHAD. No adverse event occurred in any study.	increased GI symptoms; reduced incidence kidney stones, hypoglycemia, ketoacidosis, constipation in pediatric epilepsy cohort (Liu et al, 2006)	tremor/rigidity irritability, hunger, thirst were self-reported at higher frequency in KD weeks 1-4. All sx's normalized in weeks 5-8 compared to LFD aside from excessive thirst (5/22).			
*While ketogenic diet macronutrient ratios vary between studies, the targets are similar to those used in prior KD study in PD, Phillips et al, presumably better tolerated than strict 4:1 KD, and is proposed for the current ketogenic diet study.						

Despite the limitations of low subject numbers and infrequent data capture, these previous trials of nutritional ketosis in Parkinson's suggest motor, cognitive and non-motor symptom benefit, particularly in symptoms including fatigue, depression, and urinary frequency, in comparison to high carbohydrate, low fat diets even at moderate reported ketosis values of 0.3-1.0 mM. The mechanisms and specific biomarkers of PD impacted by ketogenesis have not yet been defined, nor the time course of any effects. Furthermore, adherence as well as side effects related to micronutrient deficiency or extreme skew of macronutrient composition towards lipids remain barriers to wider adoption of studying ketogenic diet for treating PD. MCT ketogenic diet (MCT-KD) may require less stringent carbohydrate restriction and fewer micronutrient supplements than classic KD; may improve tolerability (Liu 2008); and has been shown to augment mean levels of ketosis in healthy volunteers (Harvey 2018). MCT-KD remains unexplored in PD, particularly as synergistic therapy. In this clinical study, an inpatient pilot design is proposed to study the effects of a carbohydrate-restricted ketogenic diet supplemented by MCT oil nutritional ketosis vs a standard non-ketogenic, low fat diet (SAD), within the keto-induction period and shortly thereafter over one week, on brain network connectivity via EEG cortico-cortical connectivity and motor waveform characteristics, motor physiology and clinical scale (UPDRS), cognition, autonomic function (heart rate variability, orthostatic blood pressure) and symptoms of PD with fingerstick beta-OHB levels and respiratory exchange ratio (RER) in the Metabolic Unit +/- continuous glucose monitoring (CGM) to establish which, if any, PD symptoms may be modulated by ketogenic intervention in relation to suspected or likely metabolic pathways, to inform subsequent study into symptomatic benefit and/or disease modification. To better address the primary aim of diet feasibility, a two-week outpatient segment is proposed to assess more long-term effects of a similar ketogenic diet and further estimate adherence using an online, web-based application.

2.2 Background

Sporadic PD is clearly a complex, multifactorial disorder. Several lines of evidence converge on mitochondrial insufficiency as a key factor in the pathophysiology of sporadic PD. Mitochondrial electron transport chain genes are downregulated in PD and incidental Lewy Body Disease (iLBD) at post-mortem, particularly those activated by transcription factor PGC1alpha (Zheng et al, 2010). Genetic forms of PD, LRRK2 and Parkin, involve constituents of endoplasmic reticulum (ER) homeostasis and are implicated in pathologic ER stress (oxidative stress and apoptosis) related to impaired ubiquitin-proteasome function. Furthermore, upstream impaired mitochondrial cycling produces alpha-synuclein misfolding as by MPTP toxin, with further impaired mitophagy and autophagy from lysosomal

downregulation (Colla et al, 2019, Lehtonen et al, 2019). The reactive oxygen species and damaged protein, lipid and DNA are proposed as biomarkers of PD (He et al, 2018), but the disease mechanism involves impaired mitochondrial function.

Modulation of cellular energetics has become increasingly used as a therapeutic tool (Kossoff and Hartman, 2012), and monitoring the metabolic effects can provide a biomarker of the therapy, controlling the degree of modulation. Originally studied in epilepsy, nutritional ketosis (NK) is increasingly being studied, and given positive results, adopted in neurodegenerative disease such as PD, Alzheimer's Disease, and amyotrophic lateral sclerosis that involve oxidative stress, impaired cellular metabolism, and toxic species accumulation (Bosco et al, 2006; Nakabeppu et al, 2007). Furthermore, there is the activation of NLRP3 inflammasome in both human post-mortem and animal models of PD (Wang et al, 2019). The primary physiologic ketone body, Beta-OHB, attenuates inflammasome activity in LPS treated mouse monocytes in vitro and mouse models of NLRP3-mediated disease (Youm et al, 2015). Apolipoprotein, the main protein constituent of high density lipoprotein, is found to be abnormal in PD, specifically the A1 type (ApoA1) is consistently reduced and c-reactive protein (CRP) elevated with changes found to be correlated with more severe disease in a large cohort (Oxford Discovery cohort, Lawton et al, 2020) and may also be elevated by ketogenic diet (Kwiterovich et al, 2003, Mooradian et al, 2006). Elevated CRP also was associated with worse motor prognosis in a separate PD cohort (Umemura et al, 2015). Thus, the plasma markers ApoA1 and CRP may be useful as exploratory biomarkers that may show improvement from ketogenic diet. The mechanism of action and timecourse of symptom reduction of NK in PD remain unclear, but has theoretical benefits to mitochondrial function and gene expression, as detailed below.

Ketogenesis has several proposed benefits for Parkinson's Disease in keeping with its established pleiotropic effects. There are proposed direct and indirect benefits (Veech et al, 2017). The mechanism of action and time course of symptom reduction of NK in PD remain speculative, although in vitro and animal models suggest a direct benefit of ketone bodies that parallels the fasting state in signaling cell survival and longevity. The primary mechanism for this is described as epigenetic modification by histone acetylation towards an open configuration, specifically by repressing histone deacetylase 1 (HDAC1) (Shimazu et al, 2013).. Accordingly, gene expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1A) leads to increased mitochondrial biosynthesis, and expression of transcription factor forkhead box O-3 (FOXO3) generates nucleotide coenzyme / reducing agent dihydronicotinamide-adenine dinucleotide phosphate (NADPH). According with the fasting / ketogenic state there is also increase in supply of adenosine monophosphate kinase (AMPK), which inhibits mammalian target of rapamycin (mTOR) and thereby augments autophagy. Finally, specific to PD, ketogenic researchers such as Dr. Veech hypothesized that KD would lead to increased neuron / astrocyte neurotransmitter storage and signaling from increase in mitochondrial efficiency, as ketone bodies bypass tricarboxylic acid (TCA) cycle complex I, which is especially deficient in PD post-mortem RNA study (Zheng et al, 2010), as well as increasing the redox span and free energy of ATP hydrolysis by oxidizing CoQ10 to ubiquinone and reducing NAD+ to NADH (Veech, 2004). Another effect seen as beneficial in neurodegenerative disease as well as epilepsy relates to increase in the efficiency of TCA cycle conversion of oxaloacetate to acetyl-CoA and export from mitochondria for gluconeogenesis, limiting precursor availability for aspartate and thereby increasing glutamate availability for conversion to GABA, which is consistent with reported elevated seizure threshold to current-induced seizure models in rats and elevated GABA in brain synaptosomes (Erecinska 1996); that same group also reported increases in GABA production from a glutamine precursor radiolabeling study of rats receiving beta-OHB, and CSF study of children with refractory epilepsy on the ketogenic diet (Dahlin et al 2005). Furthermore, paired-pulse TMS study of healthy volunteers on ketogenic diet showed increased short intracortical inhibition (SICI), widely attributed to GABA-A signaling as well as increased central beta power on EEG (Cantello

et al, 2007). Of potential relevance to PD, GABA is one of several neurotransmitters with deficiency by selective neurodegeneration both in striatum and in other sites including nuclei regulating REM onset, preoptic nucleus and nucleus reticularis (Brooks and Peever, 2011), GABA myenteric neurons involved in peristalsis (Grider and Markhlouf, 1992), and smell regulation by olfactory interneurons (Pirez and Wachowiak 2008). GABA agonism may be therapeutic in PD as suggested by a study of zolpidem, a GABA-A agonist, in an early, unilateral untreated PD cohort showing increase in M1 beta from the unaffected side and a reduction in pathologic beta from the affected side (Hall et al 2014) as well as GAD AAV2 gene therapy in STN at 12 months showing stable, modest reduction in UPDRS-3 motor score and reduced motor fluctuations (Niethammer et al, 2017). Neuroimaging correlates of GABA signaling may offer a biomarker of PD and potential effect of ketogenic therapy, with hypothesis that KD exerts an attenuation of pathologic hyperactivation of pallidal efferents through striatal GABA signaling, as well as potentially attenuating pathologic calcium-related oxidative stress in dopaminergic /non-dopaminergic neurotransmitter synthesizing cell groups with high metabolic rate affected by Parkinson's.

There are also potential indirect benefits related to adaptive response to decreased insulin / insulin-like growth factor 1 (IIS) signaling leading to growth factor expression, viz. Brain derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF2), which are implicated in substantia nigra dopaminergic neuron synaptogenesis and survival (Mattson et al, 2018). Plasma BDNF has furthermore been suggested as a biomarker of PD (He 2018), shown to be decreased in PD with and without depression compared to control cohorts (Rahmani et al 2019), furthermore found to be increased in plasma of healthy volunteers following 4 weeks of KD (Gyorkos et al 2019), while evidence for FGF 2 / other family types in PD and modulation by ketogenic diet remains less clear. While the relation of BDNF to PD symptoms is not clear in the literature, it is found to be a biomarker of exercise and possibly of ketogenic diet as well, thus is proposed as a KD biomarker pre vs. post intervention in PD.

Using MRS in healthy subjects, brain (occipital lobe) beta-OHB ketone values correlated well with plasma ketosis values ($r = 0.86$) with brain-plasma slope of 0.26 (Pan et al, 2000). Experimentally, the pathway of ketone body (primarily beta-OHB, but also acetoacetate) entry across BBB has been described via active transport (MCT1/MCT2) as well as diffusion. Plasma ketone values will be therefore used as a proxy for brain values.

Within the short-term, 3-week follow-up of the proposed study, there is not thought to be sufficient time adequately to test disease modification effects such as clearance of alpha-synuclein oligomers or inflammation. Nonetheless, a two-fold acute effect: i) augmentation of mitochondrial efficiency (immediate, within hours of producing brain ketosis) and ii) production of neurotransmitters including dopamine and GABA, together provide improved signaling via intact neurons within the frontostriatal network underlying predicted symptomatic benefit within three weeks, possibly within days of keto-induction, compared to a non-ketogenic, standard diet. Mitochondrial efficiency and neurotransmitters will not be tested directly, but rather indirectly via effects on systems of study, the frontostriatal network via EEG and the parasympathetic system via heart rate variability. As exploratory measures of the study, hypothetical mechanism underlying symptom improvement as above will be tested via purported biomarkers of ketogenic diet – 1) EEG beta power in central regions implicated in motor control, and 2) BDNF levels and cardiovagal function – rater-blinded to ensure objective testing of this hypothesis.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

a. <u>Risks associated with MCT oil</u>

There are no current studies of MCT oil in Parkinson's population, however several studies have been reported for Alzheimer's Disease (AD) participants at doses reported as percentage daily energy intake of 10-40% (Reger et al 2004, Henderson et al 2009, Taylor et al 2018, Ota et al 2019) which is similar to the proposed study. The achieved doses were somewhat tolerance limited to 60-80% of target doses.

Side effects were more frequently diarrhea (50%), and in a larger study of 86 AD participants randomized to receive a proprietary blend of MCT powder / emulsifier based on a Stepan product (product name AC-1202) at dose of approximately 1 tbsp vs. 66 participants in placebo group, GI events were reported in 49% of MCT group, diarrhea in 24% vs. GI events in 27% of placebo group, diarrhea in 14% (Henderson 2009). However, the GI side effects in the MCT group were reduced substantially after change in the base of dosing from water, milk, or juice to an Ensure meal replacement drink. In a study of healthy volunteers comparing MCT oil-supplemented ketogenic diet to standard ketogenic diet, there were overall less ketoinduction symptoms by daily self-report, however a higher incidence of GI upset / nausea / diarrhea (Harvey et al, 2018).

Reported lipid profile effects are variable, ranging from no effect to increase in lipoprotein levels. The variation may be due to either population studied, e.g. Alzheimer's Dementia or Healthy Volunteer (HV), or dose. No statistically significant effect was found at a dose of 1.5-3 tbsp per day in a cohort of 15 participants with AD at 3 months (Taylor 2018), whereas in a study of 18 HV participants, 11% increase in total cholesterol and 12% increase in LDL cholesterol was found at a dose of 70g (65mL, about 4.3 tbsp) at 21 days (Tholstrup 2004). It is important to consider elevated lipoprotein levels in the context of glycation status, as small dense, glycated (apoliprotein type B) LDL particles have correlated robustly with cardiovascular events, whereas non-glycated (large, non-dense) LDL has not. Any adverse lipoprotein effect from a three week study exposure is expected to be less than the other studies mentioned to be negligible.

The refined fatty acid caprylic acid (C8) from MCT oil meets GRAS FDA exemption, GRAS Reference (FEMA No.) 2799.

b. <u>Risks of ketogenic diet</u>

Risks of ketogenic diet have varied based on population, with above studies of ketogenesis involving MCT oil and other ketogenic diet studies in PD, AD, and HV groups reporting GI side effects (nausea, cramps, diarrhea, constipation) as being most common. There are also known effects of ketogenesis natriuresis that may relate to a general fatigue/malaise, headache, which have been altogether termed "keto-induction symptoms" or "keto-induction flu" that may relate in part to the dehydration as well as reduced intake of certain dietary micronutrients, particularly potassium and magnesium, produced by some ketogenic diet regimens. The goal of MCT oil supplementation is to reduce the extent of keto-induction symptoms by shortening keto-induction latency, which was in fact reported in a HV group (Harvey et al 2018). Other rare adverse events reported during ketogenic diet intervention in pediatric epilepsy population and attributed to the ketogenic diet are renal stones, vitamin D deficiency, pancreatitis, bone fractures, cardiomyopathy (due to selenium deficiency) and carnitine deficiency (Kossoff and Hartman, 2012). These side effects have not been reported in studies in the adult neurodegenerative studies reviewed and are unlikely to occur in this short-term study. Risk can be lowered by careful attention to hydration status, daily vitamin, mineral, and fiber supplementation, and vigilant monitoring of electrolytes for depletion or acidosis. However, adverse side effects of mild metabolic acidosis, vomiting and electrolyte derangement are possible and will be carefully screened and monitored and may lead to care escalation / study removal if severe.

Modest weight loss is a feature of ketogenic diet, and while this is considered beneficial in some studies of obesity/related disorders, it is not regarded as a health risk in this study (excluding BMI <22).

Specific to participants who are hypertensive or diabetic, ketogenic diet is generally regarded as safe and beneficial in lowering blood pressure and fasting blood sugar levels. However, there can be risk if this occurs and medication is not adjusted due to overshoot of therapeutic effect. Risk will be mitigated by excluding uncontrolled hypertension defined as SBP > 180 or DBP > 105, and uncontrolled diabetes (A1c > 8.0%) and monitoring blood pressure and blood glucose levels frequently, with potential reduction of glucose or blood pressure lowering medication as per discretion of those study investigators who are licensed physicians credentialed at the NIH Clinical Center.

Other procedures and measurements (venous blood draws, motor physiology, cognition, EEG recording) are expected to incur minimal risk similar to what is encountered in routine clinical evaluation.

c. Risks of neurologic examination:

There is minimal risk associated with a routine physical and neurologic examination or with PD screening questionnaires.

d. Risks of surface ECG:

Risk of skin irritation at the site of the ECG electrode may be minimal in some subjects. There is minimal risk associated with surface ECG.

e. Risks of EEG:

There is minimal risk concerning the EEG technique itself. The electrode cap can be uncomfortably tight. There may be mild irritation of the skin and scalp from the mild abrasion needed to assure good contact or from the collodion, paste, or gels used.

-if participant decides not to perform in any measurements, they will not be per se removed from the study. Options include re-testing at a later time and omitting specific declined tests.

f. Risks of video recording:

There is minimal risk to patient confidentiality. This is because video will be done only with participant's signed informed consent, including option to blur facial features. Video will be taken by NIH issued video camera kept locked in the office, and files will be transferred to secure NIH sever for later review by a separately blinded rater. Videos may be viewed for official NIH teaching / educational purposes within NIH staff. Any external sharing such as for academic conference presentation will be done only with participants' written informed consent.

g. Risks of Blood Draw

There is minimal risk of bruising or prolonged bleeding from phlebotomy; local pain and fainting or infection at the needle entry site are possible but rare side effects.

h. Risks of completing study questionnaires

The study questionnaires may be frustrating or stressful. Subject may refuse to answer any question or to stop a test at any time and for any reason.

2.3.2 Known Potential Benefits

MCT ketogenic diet has shown improved cognitive outcomes in several small AD trials (reviewed in Pinto et al 2018), and ketogenic diet reported improved lexical fluency and working memory compared to a high-carbohydrate, low-fat diet in PD cohort with mild cognitive impairment, despite low mean beta-OHB level of 0.31 mM at study conclusion of week 8 measured in the ketogenic diet group (Krikorian et al 2019). Other clinical trials, VanItallie et al 2005 and Phillips et al 2018, recorded UPDRS as primary outcome and found reduction in total scores and all sub-scores, and in the larger KD study group of 18 participants by Phillips et al, there were reported greatest improvement in UPDRS-I and less improvement in UPDRS-III compared to low-fat group (with actual more improvement of UPDRS-III in low-fat group and greater side effects of subjective tremor / rigidity in the ketogenic group, which accounted for ½ of 4 KD arm dropouts). Perhaps correlating with non-motor symptom reduction that was found, BDNF expression is increased directly by exogenous beta-OHB through NFKB activation and HDAC inhibition. BDNF levels are increased independently by exercise (Kang et al 2020) and ketogenic diet (Mohorko et al 2019), mediated by norepinephrine circuits in mice (Garcia et al 2003; Chen, Russo-Neustadt 2009) and BDNF is found to be an obligate intermediary of hippocampal dependent learning and memory as per single nucleotide polymorphism in mice / neuronal culture studies and impact on human neuropsychiatric disease (Hariri et al 2003). CREB is also increased in fasting and exercise animal models, mediating long term potentiation (LTP), which is considered a proxy of learning and memory. BDNF is also associated with brain cholinergic development and heart rate parasympathetic regulation in mice through genetic mice knockdown study (Wan R et al 2014). BDNF is reduced in PD (Rahmani et al 2019) but does not clearly correlate with PD severity. BDNF was increased in subjects with metabolic syndrome following 4 weeks of ketogenic diet as well as 4 weeks of exercise in additive fashion by 20% and 38% respectively via crossover study (Gyorkos et al 2019). Therefore, there appears to be an indirect correlation with PD as a biomarker of disease, as well as with depression, and a potential therapeutic biomarker with KD. BDNF may also be elevated therapeutically in PD via 12 weeks intensive exercise as reported in MDS poster Fall 2019 in Nice, France: "High Intensity Interval Training May Elevate Serum BDNF Levels in Parkinson's Disease Patients."

Related to mitochondrial quality, ketogenic diet, fasting, and exercise upregulate the mitochondrial deacetylase "silent information regulator 3" (SIRT3), which is found to serve multiple roles in mitochondrial integrity including mitochondrial biogenesis, protein oxidative stress response (unfolded protein response) and mitochondrial DNA repair, and also found to be active in NMDA/glutamate-induced excitotoxicity (Hasan-Olive 2019, 30027365). PGC-1alpha provides a serum marker of mitochondrial biogenesis.

In addition to the above, low carbohydrate diets are consistently associated with reduced insulin secretion, reduced hunger, greater weight loss and may produce greater energy expenditure than low-fat (high carbohydrate) diets (Ebbeling et al, 2012) although contrary studies on energy expenditure are found (e.g. Tagliabue et al, 2012). Ketogenic diets have also been associated with slight reductions in blood pressure (e.g. Cicero et al, 2015). Furthermore, there are reported reductions in dyslipidemia (reduction in triglyceride:HDL, a marker of coronary artery disease, Lemos da Luz et al, 2008) and non-alcoholic fatty liver disease (Tendler et al, 2007).

<u>Potential immediate benefits (obtained during the study) are as follows:</u>

- Increased insulin sensitivity with reduced body weight and possibly reduced triglyceride level
- Mildly reduced blood pressure, possibly involving reduced pathologic sympathetic tone

- Possible improvement in PD symptoms such as: inattention, fatigue, depression, tremor, bradykinesia, pain, urinary frequency, daily off time

Insulin sensitivity (decreased fasting insulin levels, decreased HOMA-IR) is consistently increased by NK related to increase in the gastrointestinal incretin glucagon-like peptide 1 (GLP1) signaling. Due to fuel from adipocyte stores via beta oxidation, reduction in total body weight and in abdominal fat are also consistent findings of NK.

Potential long-term benefits irrespective of continuing study intervention

- Social benefits from participating in support groups / ketogenesis or other diet and fitness interest groups
- Indirect benefit from involvement in science to better understand and treat Parkinson's Disease (humanitarian / service work)

2.3.3 Assessment of Potential Risks and Benefits

The diet has already been suggested to benefit Parkinson's Disease outcomes as per standardized clinician rating scales (UPDRS) and cognitive tests. However, the data are not compelling to adopt ketogenic diet as medical guideline given use of mostly clinician rating scale with known interrater and intrarater reliability limitations in small studies. Additionally, there are key questions that remain unanswered, such as when the benefit occurs; if benefit correlates with plasma ketosis value; if so, at what ketosis value; and whether motor or non-motor systems seem to be most responsive when tested together on the same participants serially in the short-term. The participants may benefit in terms of nutritional education through the dietary intervention and better knowledge of their disease symptoms in response to metabolic measures such as glucose, dietary changes, and close attention to on/off fluctuations via self-report. MCT oil has not been studied in Parkinson's Disease and may improve tolerability of the diet. The risks of ketoinduction symptoms and GI upset are known in other populations and so will be closely monitored, tracked and intervention adapted as needed to minimize them. Overall, the risks of this study from simple dietary changes that have been studied in other populations are expected to be comparatively low relative to disease-modifying pharmaceutical agents studied for PD. One unknown potential benefit from long-term continuation of ketogenic diet (outside the present study) would be disease modification, such as from increase in uric acid / reduction in oxidized metabolites, and by augmentation of mitophagy / autophagy of toxins such as hyperphosphorylated alpha-synuclein. As a short-term study, there is not expected to be direct long-term benefit. However, the justification in addition to scientific value of understanding ketosis in PD is an indirect benefit to participants, whereby they are expected to gain better understanding of their own diets, irrespective of study group, by working with study investigators in objective measurements of physiologic effects and receive recommendations about healthy diet.

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	OUTCOME MEASURES	JUSTIFICATION FOR ENDPOINTS
Primary		
Feasibility of MCT-KD x three weeks	Composite outcome (all of the following measures must be met):	MCT-KD has never been studied in PD population (expected to

	1) Recruitment reaches target of 32 participants or as per pre-set interim analysis 2) Study retention rate >80% of all participants at 3 weeks 3) Adherence (</= 10% mean daily energy from net carb over 2 weeks MCT-KD outpatient, assessed by 2x/week unscheduled phone calls by CC Nutrition staff) 4) Acceptability – Likert scale >/=2 (at least somewhat likely to use diet in future and at least somewhat effective in improving PD symptoms) at end of study (week 3)	augment ketosis) and feasibility has not been established.
Secondary		
Timed Up & Go (TUG) time, mean difference between groups on study day 7	Mobility on the Timed Up and Go (TUG) test, daily (fastest of three trials per session) units: seconds Range: 8-18s	TUG time is generally regarded as an index of fall risk – estimated 20% risk reduction with 4s faster time (Nocera et al, 2013)
Tertiary/Exploratory		
Nutritional ketosis (NK) improves PD symptomatology at three weeks in the following: in cognition (improved attention, recall, and executive function), and motor function (reduced akinesia and tremor). A low fat (standard American) diet (SAD) does not. NK reduces non-motor symptoms, subjective motor fluctuations and daily off time within the immediate keto-induction period, while SAD does not (mean difference at 1	Stroop word-color interference latency (unit: ms, range: 110-135 ms), 0,1,2,3 back recall task (unit i: % error of missing true n-back letter presented; range i: 0-back 0-10%, 1-back 4-20%, 2-back 15-30%, 3-back 20-32%; unit ii reaction time (RT) ms, range ii 0-back 500-740 ms; 1-back 600-980 ms; 2-back 700-1200 ms; 3-back 700-1200 ms); fingertap speed and rhythm (MN and PQ keyboard tasks; units MN and PQ keystroke speed, in keys/s; range MN	All of these tests have been shown to be impaired in PD cohorts and are at least semi-quantitative and amenable to serially repeated measures with good test-retest reliability Scalp EEG is non-invasive, easy to perform in the setting of intervention, and as

week). NK increases heart rate variability, (mean difference baseline vs. 3 weeks).	2-5 keys/s; range PQ 0.6-1.5 keys/s); latency of placing and returning to dish 9 holes on pegboard (9-HPT) units: s, range 15-45 s) Non-motor symptom scale (NMSS, total score out of 360 total) Waking ON time (daily subjective worksheets, unit: hours (h), range 2-15h), keto-induction symptoms (daily worksheets or self-report, Likert scale 0-3 for various symptoms related to ketogenesis), UPDRS-III motor score (range 0-72), heart rate variability (SDNN, units ms, range 20-130 ms), simple reaction time, units ms, range 360-400 ms), plasma BDNF 15.4 +/- 3.5 pg/mL (Gyorkos et al, 2019), Geriatric Depression Scale (GDS) normal 0-9 mild depression 10-19 severe depression > 20; Gastrointestinal Symptom Rating Scale (GSRS) Avg total score 1.50-1.55; EEG premotor cortex (M1) waveform characteristics (unitless ratios, asymmetric steepness, rise:fall or steepness ratio range 0.025-0.15 and peak:trough or sharpness ratio, range 0.01-0.075 (that were shown to have high correlation to phase-amplitude coupling, which is also a unitless measure by modulation index, range 0.01-0.037) . Possible measurement of quantitative EEG (qEEG) power spectra mv^2/hz qualitatively assessed vs. normative distribution (high, med, low), phase lag index (0-1) and weighted network cluster coefficient, path length by frequency band	shown to provide a biomarker correlating with PAC (which correlates with UPDRS), if validated in this study will be a relatively reproducible neuroimaging test for future studies. ON/OFF symptom computer-based survey daily assessment (PsyToolkit) will help to further show correlation of PD symptoms with ketosis values Non-motor symptom severity (NMSS) scale and Geriatric Depression Scale (GDS) are NINDS Common Data Elements for PD Effect of KD on UPDRS included for consistency with existing literature HRV / cardiovagal responses to Valsalva / deep breathing show autonomic function. It is unknown if ketogenic diet will reduce deficits in these tests in the acute period.

4. STUDY DESIGN

4.1 OVERALL DESIGN

The experiment will take place predominantly in the Metabolic Unit, 7SW-S (1-week inpatient) and at home (2 weeks outpatient). Additionally, EEG, TUG, and heart rate variability tests will be performed in the HMCS labs within the 7SW-Neurotesting area.

This study is a single center (NIH Clinical Center), phase 1 feasibility/tolerability assessment of MCT oil subtype of ketogenic diet in a PD cohort, randomized, double-blinded vs. standard American (high-carbohydrate) diet based on macronutrient percentages of calculated daily energy expenditure using measured pCO2 and pO2 and Weir formula for indirect calorimetry, performed on screening visit, to give caloric macronutrient ratios 50-55% carb, 35% lipid, 10-15% protein in SAD group and 5-10% carb, 80% lipid, 10-15% protein in MCT-KD group on the 7SW-S Metabolic Unit with assistance of CC Nutrition Department staff. Consent, baseline lipid panel, BMP, and pregnancy test will be done at screening visit. Randomization will occur upon screening visit, stratified by age >=65 or <65 and baseline TUG score >= 11.5 s or <11.5 s. Protein macronutrient percentage of individual calculated daily energy intake will be the same between the two groups. Diet will be within subject factor for both groups, as pre-diet intervention baseline testing is planned for hospital day #1. The potential confound of changes in activity/exercise level while in the hospital on physiologic and clinical outcomes is recognized. To address this, study investigators will form an individualized exercise plan incorporating baseline exercise and activity levels to involve stationary bike at target HR based on age-adjusted HR max target using Tanaka's formula validated in the elderly population, 208-0.7 × age, in order to approximate each participant's daily baseline intensity levels from questionnaires.
Ketosis values in the MCT-KD group will be correlated indirectly with respiratory quotient and continuous glucose monitor (Freestyle Libre Pro Glucose Monitoring System, continuous glucose monitoring (CGM) system, FDA approved for blinded professional use in clinics). The hypothesis is that nutritional ketosis will improve mobility and also motor and non-motor symptoms through a direct, homeostatic return of frontostriatal signaling by mitochondrial restoration that will occur within hours of reaching ketosis on inpatient day 3, and through an indirect mechanism of increased dopamine / GABA availability (reduction in pathologic glutamatergic hyperexcitability) that will develop more gradually, over several days.

Care will be taken to reduce bias through blinding rater(s) to intervention group ketogenic or non-ketogenic and by blinding participants to intervention or control diet given, with randomization scheme kept only by a study investigator not involved in any of the ratings or measurements (study biostatistician) and by using video rating (aside from muscle tone) of UPDRS-III by the average of AI and another movement disorders specialist outside the study, the latter rating scrambled in random assortment with respect to intervention day. Study duration will be three weeks, and total time for participants approximately four weeks including initial study visit and period of at least one week baseline prospective diet, activity and ON/OFF PD symptom fluctuations self-report, and for baseline diet collection also using unscheduled twice weekly phone calls by study nutritionist / investigator (non-rater). By the time of discharge, participants will receive daily suggested ketogenic diet meal plans for the upcoming two weeks of diet at home. Assessment of MCT-KD adherence will be made by twice weekly unscheduled phone calls by NIH CC Nutrition staff (LAI). There is anticipated accrual of up to two participants admitted at once. MCT oil from Liquigen® (KetoCal) will be given at 25% calculated daily lipid energy expenditure, similar to prior studies (refer to Section 2.3.1 for details of prior studies using MCT oil).

While a longer duration ketogenic diet study, e.g. longer than 8 weeks that are the current limit of study follow-up in PD, would address a key gap in the literature on long-term outcomes and potential disease modification, there are other gaps that can be addressed via an acute, short term study, such as target ketosis value, relation of ketosis to symptoms and the time course, tolerability of novel diet adaptations (MCT oil), and biomarker of the therapy such as EEG. These gaps altogether appear to be well addressed by an inpatient trial, a unique resource of the NIH Intramural Research Program, and altogether provide an opportunity for a contribution to the field of PD therapeutic research. Additionally, given the constraints of metabolic inpatient study, with an estimated predominance of short-lived adjunctive therapeutic benefits rather than long-term benefits, recruiting for a longer duration of inpatient stay would likely be more difficulty to reach target recruitment; more difficulty to carry out as the study measurements are to be predominantly performed by one rater (AI); and difficult to justify, amounting to a phase 2+ trial when a pilot, phase 1 trial appears important to do first in order to test the therapy in a rigorous fashion. Therefore, an inpatient/outpatient three-week study is proposed to conduct first in order to address the feasibility and time course questions.

A recognized potential confounder of assessing the effect of ketogenic diet on Parkinson's Disease symptoms is possible effect on levodopa / dopaminergic (DOPA/DA) medications. Literature search using PubMed and Google search engines shows some studies of effect of KD/modified Atkins diet on plasma AED levels ranging from no effect (Dahlin et al, 2006; Coppola et al, 2010) to modest reduction in some plasma AED levels (Kverneland et al, 2015; Heo et al, 2017), however no relevant pharmacokinetic data for effects of ketogenic diet on DOPA/DA metabolism. The fed state with light breakfast (% lipid not specified) was found to slightly increase immediate release Sinemet bioavailability compared to overnight fasted state in healthy volunteers coinciding with delayed gastric emptying with no clear effect on peak concentration in the small study (Wilding et al, 1991). By contrast, pharmacokinetic study in healthy volunteers of the effect of a mixed 38% fat, 52% carb, 11% protein meal (1046 kcal) on single dose of a dual rapid/slow release formulation of levodopa + COMT inhibitor benserazide (Madopar) showed reduction of plasma levodopa peak concentration by 32% (2.09 to 1.41 mg/L) and prolonged time to peak by 2 hours (3.1h vs 1h) with however no effect on bioavailability by total AUC (Crevoisier et al, Eu Jnl Pharmac and Biopharm 2003;55). Another levodopa formulation, Numient in preclinical study (Europe European Medicines Agency / CHMP / 672104 / 2016) similarly showed dietary fat delayed and reduced plasma levodopa peak concentration without affecting bioavailability. Similarly, in a study of single dose extended release formulation of carbidopa-levodopa (Rytary) in healthy volunteers in either overnight fasting or post-high-fat, high-calorie breakfast conditions, the initial rise in concentration of levodopawas delayed by 2 hours, peak concentration was reduced by around 21%, and total AUC was modestly increased by 13% (Mittur A et al, 2017 28236251).

Protein is well-established to affect oral bioavailability and pharmacokinetics of levodopa through shared transporters with large, neutral amino acids and clinical studies, as outlined and referenced in the protocol, and is not considered to be a factor between the two groups given that dietary protein will be controlled over a small range (10-15% total daily energy). Importantly, as confirmed in multiple studies including the above, the fed state itself introduces delayed gastric emptying, and the diet used in the control arm, SAD while not ketogenic is not low fat at 35% daily energy, thus also would reduce peak concentration as per above studies. Another variable in effect of meals on oral pharmacokinetics including PD medications is the formulation, as solid meal boluses delay gastric emptying and more likely impact peak concentration than solutions and suspensions such as proposed in this dietary study, the same in both groups.

Therefore, while literature search reveals no pharmacokinetic studies on the effect of ketogenic diet on levodopa or other medications indicated for Parkinson's Disease, inference from studies of dietary fat as

above would impute lower and delayed peak concentration (potentially sub-threshold for therapeutic window, especially in more advanced disease such as defined by Hoehn & Yahr stage 4) and therefore potentially bias towards the null hypothesis. To better account for effects of diet on levodopa metabolism, plasma dopamine will be measured twice daily at the time of blood draw for beta-hydroxybutyrate on inpatient study days 1 (baseline, pre-dietary intervention) vs. days 2-3.

Independent variable: The effect of intervention MCT-KD vs. standard diet control group (see section 2.1 table for macronutrient ratios under KD and SAD, respectively) will be measured in three ways: a) twice daily phlebotomy beta-OHB levels to be drawn #1 in fasted morning time and #2 in the evening 2 hours after dinner b) daily respiratory quotient (pCO2:pO2) looking for stabilization of decline to the 0.7 – 0.8 range, and c) continuous glucose monitor (CGM) in Metabolic Unit, looking for fluctuations, possibly correlated to symptoms, as well as trend of stable reduction < 100 mg/dL.

The interventional food-based product, Liquigen® (KetoCal), will be administered by NIH CC Nutrition staff to MCT-KD group participants as per defined blinded diet.

Primary Outcome: feasibility will be assessed in a composite fashion by retention rate >80% at study end (week 3) ; study adherence in the outpatient segment defined by maintaining mean carbohydrate limit </= 10%, as by CC nutrition staff twice weekly unscheduled calls data input to online application); reaching recruitment target of 32 participants, unless otherwise defined by interim analysis (see Section 9); and acceptability by likelihood to use the diet in the future and effectiveness in reducing PD symptoms both "somewhat" >/=2/4 on Likert scales. All criteria must be met.

Secondary outcome: Timed Up & Go (TUG) time at between MCT-KD and SAD groups mean difference on inpatient day 7.

Exploratory outcomes:

Effort will be taken in establishing medication and testing schedules, for outcomes to be assessed at the same time from last dopaminergic medications and other psychotropic medication administrations including cholinergic and muscle relaxants (see table 2). All exploratory outcomes will be assessed pre (admission day 1) and post (day 7 and week 3) diet intervention. Additionally, reaction time, Stroop test, N-back test, MN/PQ fingertapping tests, 9-HPT, UPDRS-3, and resting HRV will be tested daily on admission days 2-6.

Stroop word color interference latency (Stroop test B), N-back test (3-back letter), MN and PQ keystroke tasks, tremor accelerometry, 9-hole pegboard test (9-HPT), and simple reaction time (SRT), EEG waveform analysis (steepness ratio, sharpness ratio, modulation index), and quantitative (q)EEG assessment; daily assessments of heart rate variability (SDNN, pNN50, rMSSD, power spectral analysis), and supine to standing blood pressure and heart; resting heart rate (5 min recordings daily), UPDRS including daily UPDRS-3, daily ON time, daily PD symptoms, daily keto-induction symptoms, pre vs. post intervention Non-Motor Symptom Severity Score (NMSS)

All testing to be done by AI, at a certain scheduled time daily or specified testing schedule with respect to last meal and dopaminergic medications or others potentially modulating neuromuscular system (see Table 2 description for list). If patient has DBS, the settings will not be adjusted unless there is the impression of study investigators including PI who are movement disorders specialists that a significant motor complication has occurred during the intervention that might be improved by DBS adjustment. For

example, dyskinesia has been reported in prior ketogenic PD trial (Phillips et al, 2018) and could be improved by reducing DBS stimulation. For more details on adverse events please refer to Sections 9.5-9.6.

Table 2 – flowchart / schedule of testing

Care will be taken to minimize participants' test/retest variability from psychotropic medications. Accordingly, medications from the following classes will be taken up to the night prior to testing (held for testing): anticholinergics, NMDA antagonists, dopamine blocking agents, muscle relaxants, anticonvulsants, GABA agonists. Tests will occur in the same order; and care will be taken to control timing of AM dopaminergic dose in relation to testing, providing instruction for participants to take their dopaminergic medications at a set 30 min prior to breakfast. Morning dopaminergic medications will be taken in the overnight fasting state to minimize group differences in pharmacokinetics related to diet, as outlined in section 4.1 above. For each participant, testing will be done in a single session lasting approximately 1.5-2 hours, and time from dopaminergic medication dose to end of testing will be around 2-3 hours. No changes will be made to dopaminergic medication prescription, unless clinical emergency as per credentialed clinician assessment. If participant's levodopa or agonist dose interval requires repeat dose during testing, we will make no changes but maintain the same dose schedule throughout the testing days 1-7 and week 3.

Test, meal, medication	Duration	Approx. time from DA Rx (hr:min)
Fasting BOHB (days 1-7, week 3) and plasma dopamine** (days 1-3 only)	(as per CC, ~7AM)	~12 hours
Dopaminergic medications, L-DA / DA	(fasting) 7:30-8AM	~12-13 hours
Breakfast (pureed shake)	(~8-8:30)	~30 min
TUG	20 min	~1 hr
(rsEEG + HRV maneuvers) *(day 1, 7, wk 3 only)	30 min*	~1:20
9HPT	10 min	~1:20 (1:50*)
MN / PQ tests	8 min	~1:30 (2 hr*)
SRT / CRT	8 min	~1:38 (2:08*)
Stroop test	8 min	~1:46 (2:16*)
(3) n-back test	8 min	~1:54 (2:24*)
HRV daily	8 min	~2:02 (2:32)*
UPDRS-3	10 min	~2:10 (2:40*)

Abbreviations:
levodopa (L-DA),
dopamine agonist (DA),
Timed Up & Go (TUG),
9-hole pegboard test (9HPT), simple / complex reaction times (SRT/CRT),
heart rate variability (HRV)

1:30 - 1:45 total + 30-60 min (breakfast) → up to 3 hr total time from last dose

Note: The times listed on the chart are the target timepoints, and while every effort will be made to keep testing as close to the target time as possible, it is expected that there will be some variation in actual testing times. Times will be recorded. Variation in testing times is expected and will not be considered a protocol deviation. Home dose of dopaminergic medications will be given in the morning of the testing in the fasted state approximately 30 minutes prior to breakfast, allowing for at least 1 hour after dose prior to the start of testing to decrease variability in absorption.

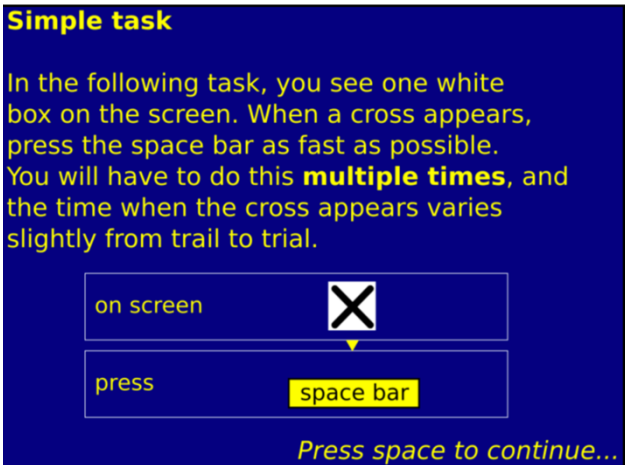
<u>Mobility assessment: Timed Up & Go (TUG) test</u>

To test the hypothesis of improvement occurring acutely (within days of reaching ketosis) due to improved mitochondrial function and neurotransmitter signaling, TUG will be tested by AI between group mean difference on study day 7 to target TUG power analysis (see section 9 for details). Pre-intervention screening visit time >/= 11.5s or <11.5s will be used as covariate for group randomization. Power analysis was chosen for TUG as a measure showing good intra-rater reliability (Dal Bello-Haas et al, 2011) and correlated with fall risk (Nocera et al 2013), hence meaningful for motor function.
TUG will be done in the hallway of 7SW Neuro testing with distance of 10 ft demarcated by tape, using chair and stopwatch, best of three trials, daily as per table 2 testing schedule above.
To control for biological / Levodopa variability, all secondary/exploratory tests will be done at approximately the same time with respect to dopaminergic Parkinson medication and other psychotropic medication dose and at approximate same time of day and same order of tests. Additionally, plasma levodopa will be measured twice daily at the times defined for BOHB blood draw.

Simple reaction time

Simple reaction time has been consistently shown to be impaired in PD (Flowers 1978; Evarts et al, 1981), and the increased latency is found to correspond to motor symptoms of tremor and rigidity in patients with asymmetrically lateralized symptoms and to be furthermore increased bilaterally in patients with bilaterally symmetric symptoms (Yokochi 1985). SRT also is found to be improved after levodopa therapy (Zappia 1994). Furthermore, SRT has been compared to complex reaction time (CRT) and it was found that the same degree of deficit was found in both, suggesting that the deficit is not a function of complexity (Sagar et al, 1992), as has been reported elsewhere (Jahanshahi, Brown, Marsden 1993). SRT is therefore considered to be a potentially useful test of motor benefit related to hypothesis of increased neurotransmitter availability and signaling related to ketogenic diet, with improvement in reaction times on the diet but not on a non-ketogenic, healthy diet.

As per pre-determined scheduled time, the subject will be asked to sit comfortably before a computer screen and look at the white box in neutral fixation space, and when ready to press spacebar with less affected or dominant thumb on a keyboard as soon as seeing an X appear within the box; 8 practice repetitions will be followed by 20 test repetitions. Immediately afterwards, subject will be asked to look at a row of 4 white boxes and press one of the following keys, z x , (using fingers 2 and 3 bilaterally) corresponding to the X within the box, 8 practice followed by 40 test repetitions. Testing will occur daily.



https://www.psytoolkit.org/c/3.0.0/run_exp?name=Simple_and_Complex_RT

Stroop Word-color interference test
At pre-determined scheduled time as per Exploratory Outcome heading, from 40 stimuli, colored (red, green, blue, yellow) number words will be displayed on a computer screen, and the difference in latencies between incongruent (color and word of the color different) congruent (color and word of the color same) time to correct response will be recorded, a minimum of 20 correct responses. The % correct from total responses will also be recorded. Following is URL to perform the test:
https://www.psytoolkit.org/lessons/experiment_stroop.html

As explained in the software instructions, the fixation in PsyToolkit occurs for 200 ms, and then the colorword trial is shown for 2s - the program records correct and incorrect (or no keystroke) responses separately and averages the latencies over 40 trials.
Testing will occur daily.

Stroop word-color interference is to be tested on day 1, week 3 final visit, and daily on the same testing schedule as a function of the intervention over 7 days.

N-back task
As per pre-determined scheduled time, 3-back task will be tested at a certain time daily with letters A,B,C,D,E,H,I,K,L,M,O,P,R,S,T presented for maximum 2000 ms, at a rate of new stimulus ever 2500 ms, for 20 trials.

If the stimulus is the same as 3 trials ago, participants press the 'M' key. If the stimulus is not the same as 3 trials ago, participants press the 'N' key. (M for memory, N for No)
At the end of 20 trials, error % is given for missing 3-back items, and reaction time (ms) is calculated.
A demo of the test is found here:
https://www.psytoolkit.org/experiment-library/experiment_nback.html
Testing will occur daily and at week 3.

MN and PQ keyboard tasks
As per pre-determined scheduled time, testing to occur based on a computer system developed at NINDS 7SW Neuro Testing area (Patrick McGurrin, PhD Neurophysiology), with software prompt to begin tapping back and forth between M and N as rapidly as possible within 30 seconds, and the number of taps are recorded as well as the time stamps on the computer processor, subsequently prompting the next test to alternate between the P and the Q keystrokes as rapidly as possible, counting #seconds for 30 repetitions and time of each keystroke also recorded. Each task is repeated three times per arm and averaged. Thus, speed (correct repetitions / minute) and rhythm (keystroke vs. time) can both be plotted. This will be helpful to assess bradykinesia and dysrhythmia of Parkinson's Disease as a function of the intervention, in daily testing over 7 days and at week 3.

9-hole pegboard task (9-HPT) (Rolyan A851-5)
As per pre-determined scheduled time, testing to occur as per this commonly employed measurement in clinic / rehabilitation specialists such as occupational therapists to assess finger dexterity. Normative data has been established (Mathiowetz et al, 1985). This consists of a square board with 9 holes spaced 1.25 in apart, each hole ½ depth and drilled with a 9/32 in drill bit, next to a container for the pegs. In Parkinson's Disease, means of 26.6s to 36.7s were found at Hoehn & Yahr stages 2-3 in dominant hand, and 27.5s to 36.8s in non-dominant hand (Earhart et al 2011). SEM was found to be 1.02 s for dominant hand and 0.82 s for nondominant hand, indicating good test-retest reliability, to be tested daily as a function of the intervention over 7 days.

The test (Rolyan A851-5) is done with dominant arm first, with stopwatch giving one practice trial per arm provided to the timing, starting when participant touches the first peg and stopped when patient places last peg in the hole. Participants will be instructed that, if a peg is knocked from the tray or hole onto the table, they should replace it themselves according to the test phase (in vs. out of the holes), and if it is knocked off the table, the rater will return the peg to its place of origin. This is to minimize potential timing variability. (Instructions adapted from www.rehabmeasures.org, test instructions derived from Mathiowetz et al, 1985). Testing will occur daily over 7 days and at week 3.

Geriatric Depression Scale (GDS)
Given some literature on ketogenic diet and mood interaction and proposed clinical use as therapy for mood disorders (reviewed in Brietzke et al, 2018), depression will be screening using GDS, a validated instrument listed under Common Data Elements for Parkinson's Disease. The survey will be self-administered pre (screening visit) and post (day 7, week 3 visit) intervention.
Available at: < https://web.stanford.edu/~yesavage/GDS.html >

EEG waveform analysis / qEEG assessment (7 SW Neuro testing area)
As per pre-determined scheduled time, EEG recording (Brainproducts, not FDA approved) will be done as following: 64 EEG electrodes will be positioned according to 10-20 International System, with every impedance kept under 5 kOhm and single earlobe used for reference. EEG data will be amplified, filtered (DC-100 Hz), digitized at 1000 Hz (Brain Products GmbH, Gilching, Germany), and stored on a computer for offline analysis. To keep track of common artifacts, to monitor blinks and eye movements, electrooculogram (EOG) will be recorded from two extra electrodes near the right eye on the supra-orbital ridge. To monitor the heart activity, electrocardiogram (ECG) will be recorded from two extra electrodes, one under the collarbone on the right shoulder, and another one on the abdomen on the lower left side. Subjects will be asked to enter a relaxed, wakeful state minimizing movements, first 5 minutes with eyes open and then 5 minutes with eyes closed. The following metrics will be analyzed:

Spectral analysis (quantitative EEG) will be assessed in MatLab (EEGLab application) after pre-processing (removing artifacts, re-referencing) by Fast Fourier Transformation (FFT) to give frequency vs. time analysis and derive power per band, focused on: theta (4-8Hz), alpha (8-13 Hz,), and beta (13-30 Hz, also divided into slow beta 13-20 Hz and fast beta >20 to 30 Hz)), particularly investigating theta power as a correlate of cognitive impairment (reviewed in Geraedts et al, 2018). Additionally, elevated spectral power across frequency bands was found centrally and decreased posterior alpha power with slower alpha dominant rhythm was reported in a mixed phenotype PD cohort compared to controls (Moazami-Goudarzi et al, 2008). It is of interest in the proposed pilot study to attempt to replicate these spectral changes in PD as a putatitive biomarker of compensation and elevated coherence in the frontostriatal network with reduced parietal coherence, as has been described via functional MRI at HMCS. It is an exploratory aim to assess if these changes are potentially amenable to modification by ketogenic diet through GABA or other neurotransmitter effects.

In addition, it is planned to analyze the frequency vs. voltage^2/frequency for two questions:

- is there abnormal slowing (e.g. loss of posterior alpha rhythm) that is detected initially and normalizes post-intervention;

- Is there a change pre vs. post intervention in central (peri-Rolandic) beta power, reportedly increased by ketogenic diet in healthy volunteers (Cantello et al, 2007) and decreased centrally in the lesional

hemisphere post-zolpidem dose in a PD cohort (Hall et al, 2014), presumably due to elevated GABA tone as an effect of ketogenic diet.

EEG is to be tested pre/post intervention on study days 1, 7 and week 3.

Functional connectivity will be tested by frequency specific (via FFT) weighted minimal norm estimate (WMNE) for time and phase synchronization by phase locking value method for connectivity strength, a quantitative measure of phase (ranging from 0 to 1) (Hassan et al, 2014) that has been modeled via graph theory network with decreased local integration measures in alpha1 band in PD-MCI group compared to PD-CN and Control groups (Utianski et al, 2016). EEG connectivity measure of coherence can be assessed via magnitude squared coherence (inputting both phase and amplitude of electrode pairs, and by phase lag index (PLI), which calculates asymmetry of distribution of instantaneous signal phase differences between two signals (brain regions), ranging from 0 (uncoupled or phase locked) to 1 (perfect coupling, non-zero, non- π modifier). There is a consistent finding of reduced PLI in the alpha 1 (8-10) and alpha 2 (>10-13) bands broadly distributed and increased PLI in theta bands in temporal regions associated with PD-MCI compared to PD with normal cognition (Chaturvedi et al, 2019). Therefore, assessing PLI per frequency bands in defined regions is of interest as exploratory measure of the ketogenic diet intervention (pre vs. post intervention), with hypothesis of increased alpha PLI at 1 week post-intervention in the ketogenic diet group, sustained at 3 weeks.

Additionally, waveform analysis will involve metrics of M1 slope and amplitude at defined intervals surrounding each extrema (e.g. 3 point before/after) to derive sharpness ratio (amplitude peak to trough) and steepness ratio (slope $_{rise:fall}$) correlated with pathologic phase-amplitude coupling in motor striatocortical loops as per Jackson et al, 2019.

Heart rate variability

As per pre-determined scheduled time, subjects will receive monitoring daily on study days 1-7 for 5 minutes using Elite HRV finger monitor and Android application in relaxed waking state in the reclined seated position. Additionally, on study days 1, 7, and week 3 (pre/post intervention), at the time of sessions with rsEEG (see table 2 for testing schedule), trials of Valsalva Maneuver (VM) and deep breathing (DB) will be conducted as follows: VM with coaching to blow into mouthpiece attached to manometer to pressure of 40 mmHg x 15 seconds, and the recording of heart rate cardiovagal gain over the subsequent 1 minute (max RR / min RR interval); average of 3 trials will be used for analysis. DB will occur for 1 minute with 6 cycles per minute, average of 2 trials. Recording for cardiovagal testing will be conducted study day 1, 7, and week 3 via ECG electrodes using Natus Neurology machine (not FDA approved). Analysis will be done using EliteHRV and Biopac Acqknowledge software to extract using artifact removed R-R wave (N-N) intervals SDNN, pNN50, rMSSD, and power spectral analysis (LF, HF, VLF, LF/HF ratio).

Orthostatic vital signs

Orthostatic blood pressure and heart rate will be recorded daily, study days 1-7 and week 3 - supine for 10 minutes or more, then standing immediate followed by standing at 3 minutes.

UPDR parts 1, 2, and 4 will be tested pre vs. post intervention (day 1, week 3). UPDRS-3 will be assessed daily, similar time each day and relation to medications in ON state, with video recording. UPDRS-3 measures to be assessed by board certified neurologist, either directly by AI or rated by blinded rater not affiliated with the study

ON-time, PD symptoms, and keto-induction symptoms will be captured daily via online survey provided / collected to participant daily to check PD / keto symptoms and daily waking hours in the "off" state without effect of dopaminergic medications. Psytoolkit URL: **https://www.psytoolkit.org/c/3.0.0/survey?s=RQFaX**

Pre vs. post intervention non-motor symptom severity scale (NMSS, Chaudhuri et al 2007, validated by MDS) will be tested for presence/absence of fatigue, pain, urinary disturbance, and other non-motor symptoms (day 1, week 3).

Gastrointestinal Symptom Diary: to be administered daily (days 1-7), designed by International Foundation for Gastrointestinal Disorders. URL: https://www.iffgd.org/publications-library/library/341.html

Gastrointestinal Symptom Rating Scale (GSRS): Developed for dyspepsia / IBD, normative values available in population study (day 1, 7, week 3). J. Svedlund, E. Dimenäs, I. Wiklund 1995

Metabolic labs (venous phlobotomy)– baseline (day 1) vs. post-intervention (day 7, week 3) A1c (baseline, week 3), lipid panel*, insulin, HOMA-IR, cortisol, TSH, T3, free T4, CRP, Apolipoprotein, BDNF; twice daily (at pre-determined times with respect to medications and meals) BOHB (days 1-7) and dopamine (days 1-3)

*lipid panel drawn at screening, day 7, and week 3

Ketogenic diet has been consistently linked with increase in insulin sensitivity, modest reduction in glucose levels, increase in brain and plasma BDNF levels, and has been shown to reduce plasma triiodothyronine (T3) hormone levels with unknown significance.

Bioelectric impedance pre vs. post intervention (Screening and week 3): This provides an indirect measure that correlates with total body adiposity, and will be performed in the Metabolic Unit to assess if the weight loss expected with dehydration may in part be due also to reduction in adipose tissue from beta-oxidation.

Exit survey Likert scales (0-4) for likelihood of using the diet, with or without MCT oil, and effectiveness in symptom control, with space for comments on which, if any, symptom(s) responded best.

PsyToolKit URL: **https://www.psytoolkit.org/c/3.0.0/survey?s=2SZqS**

4.2 Scientific Rationale for Study Design

The core rationale for the inpatient portion of the study is to provide more depth of analysis as well as the increased monitoring than is possible in outpatient settings, to investigate whether PD symptoms are still impacted beyond placebo, and if impacted in the several days following keto-induction as opposed to at three weeks. Corollary to these aims and underlying the pilot design and primary objective, adherence will be ensured rigorously in the inpatient setting, and feasibility of inpatient dietary intervention for PD will be assessed in comparison to the more realistic outpatient setting. Outpatient comparison is used to allow for greater time for possibly delayed therapeutic effect as is postulated due to epigenetic effects related to histone deacetylase complex inhibition and other metabolic-related changes gene expression, in which one week may be inadequate whereas three weeks offers a better window. Outpatient comparison will also allow for useful consideration of feasibility of the diet in real-world setting for consideration as therapeutic adjunct.

The mechanisms and specific biomarkers of PD impacted by ketogenesis have not been defined, nor the time course of any effects. Furthermore, adherence and tolerability as well as side effects related to micronutrient deficiency or extreme skew of macronutrient composition towards lipids remains barriers to a wider adoption of studying ketogenic diet for treating PD. Acute metabolism switching via standard ketogenic diet supplemented by MCT oil as described by Phillips et al in healthy volunteers as an alternative means of inducing nutritional ketosis remains unexplored in PD in terms of the relation of different ketosis values to symptom measurements over the keto-induction period in comparison to usual diet. Given the lack of data on ketosis value in PD and mechanism, a pilot study appears useful to assess novel adaptation of MCT oil and exploratory biomarkers EEG, HRV, and BDNF. While there is a knowledge gap for the mechanism of purported acute benefits of nutritional ketosis in PD even within one week that motivates this study, there are additional hypothesized benefits that would appear only on a longer time scale for which this study would be a pilot for future, phase 2+, larger scale and longer study duration in the outpatient setting to study effects of therapeutic diet on disease progression. Such disease markers might include decrease in oxidation protein products (AOPPs), oxidized lipid metabolites and urinary nucleotides, increase in autophagy via CSF markers, and increased brain perfusion without increase in FDG-PET uptake, as was reported in recent ketogenic diet trial in AD by Neth et al 2020 at Wake Forest. It would certainly be of interest to conduct a ketogenic trial for longer duration than the other three pilot studies to test for disease modification. On the other hand, acute effects also require further study and are the aim of this proposed pilot study to establish feasibility and biomarkers of therapy in relation to the disease.

PD motor pathology of rigidity and bradykinesia is clearly associated with pathologic hypersynchrony in striatal-frontal beta oscillation along with abnormally long bursts of beta activity (Brown and Williams, 2005), and the severity of motor symptoms correlates with phase (beta frequency) amplitude (gamma frequency) coupling in motor cortex and STN (van Wijk et al, 2016), which are ameliorated with DBS (de Hemptinne et al., 2015) as well as L-DOPA (Cole et al, 2017). Also clear in the pathobiology of PD, parasympathetic and sympathetic nodes of the autonomic nervous system are involved with impaired functional testing, which can precede motor symptoms (Goldstein 2014).

Proposed acute effects of ketogenic diet in relation to symptom benefit may include increased parasympathetic tone seen in animal studies of KD related to production of BDNF, which can be directedly tested, and increase in peri-Sylvian beta power on EEG, reported in healthy volunteers on KD as a correlate of increased SICI / cortico-cortical GABA-A signaling (Cantello et al 2007), particularly relevant for PD with morbidity of depression and GI disturbances implicating non-dopaminergic neurodegeneration, for which GABA deficiency and related calcium overload are hypothesized mechanistically (reviewed in Blaszczyk 2016). The acute effects of the intervention are putatively related to increase in GABA tone, for which EEG and parasympathetic testing appear to be promising as acute biomarkers of the intervention.

The diet intervention duration of 3 weeks is limited to assess tolerability and is too short to observe effect on disease progression. However, the purpose is to provide a pilot feasibility study while generating time course in the inpatient setting for symptomatic effects and possible mechanism via EEG spectral power and connectivity, as a forerunner to long-term study. The significance of this clinical trial as being a pilot study is that while it is designed to test feasibility as well as symptomatic effects and biomarker signal within the acute period, it is contingent upon a future study that would provide a longer time period and ideally study more subjects to validate the feasibility of the approach adopted in this intervention in both symptoms and disease modification. While ketogenic diet is validated as being therapeutic in certain types of epilepsy and considered useful for many types of epilepsy by epileptologists, and the approach is

often to prescribe the diet for a period of time such as 6 months or 1 year because the therapeutic benefit of seizure reduction/cessation often endures long after switching back to a regular diet, such data are not available for Parkinson's and will require further study, which this study does not attempt to address. Rather, this study proposes to address what level of ketosis can be achieved through an MCT oil supplemented, monitored diet with less stringent carbohydrate restriction and therefore presumably better tolerated as a long-term therapy; whether there may be particular biomarkers to indicate success of the diet, such as EEG central beta power, heart rate variability or BDNF; if side effects of MCT oil are tolerable in this population with known gastro-intestinal paresis; and what effect does a given ketosis value have compared to another on serially repeated, validated measurements in PD.

Anecdotally, PwP adhering to ketogenic diet or other low-carbohydrate diets such as Paleolithic diet have reported that both diets, avoiding carbohydrate and large amounts of protein, improve symptoms such as walking speed, tremor and fatigue, even at low or undetectable values of ketosis as measured from a ketone meter reagent strips, a validated method for beta-OHB levels 0.1 – 6.0 mM (for further details of community engagement please refer to section 5.6). Accordingly, the mechanism underlying benefits of a low carbohydrate diet appears to be more multifaceted than simply ketosis, potentially involving differences related to fatty acid metabolism, levels of PUFA (polyunsaturated fatty acids), glycolysis, insulin sensitivity, levels of antioxidants, DNA repair, and buffers for coenzyme activation. This remaining question drives the study, with the question of "do ketosis values matter," which is well posed for inpatient metabolic unit study.

Of methodological importance, a learning effect is expected in repeated tests, more so in the proposed tests involving preparation and decision such as Stroop word-color interference, TUG, and 3-back recall. However, the underlying assumption of learning effect is that it is expected to be equivalent in both randomized study conditions, and moreover is of study interest for between-group effect though not directly measured. The tests were selected as ones with established reliability of repeated testing, which is operationally validated for simple motor tasks - simple reaction time (18 trials, Hamsher 1977), 9-hole pegboard test, MN-PQ keyboard tapping speed, and TUG. On the other hand, there is greater potential learning bias in cognitive tasks, particularly visuoverbal 3-back working memory task (Soveri et al 2018) where test-retest reliability has been reported as moderate, whereas simple reaction time reliability is much higher. Nonetheless, it is the aim to adjust for learning effect by randomization between the groups stratified by age. Learning effect could account for some of the difference in performance between the groups particularly in cognitive tasks. The goal is to capture which types of testing improve, whether ones more (n-back test, Stroop word-color interference) or less (reaction time, tapping speed, TUG) subject to learning effect, to infer appropriately whether or not learning effect may be considered to be operative in any difference between the groups.

The combination of inpatient and outpatient parts of the study involves some degree of discontinuity of the diet, at least in terms of monitoring but also in the diet itself. The latter difference is due to the desire to maintain double-blinding in the inpatient part of the study to minimize placebo effect, while also wanting to assess feasibility of a diet that is thought to be better tolerated than traditional, strict 4:1 (4 grams lipid to 1 gram carb + protein) ketogenic diets. The inpatient diet is predominantly liquid via blended nutrients to facilitate the blinding, but this is not practical to recommend for long-term outpatient therapy. Furthermore, while there is possibility to maintain a control group in the outpatient part of the study, it is felt preferable to prescribe ketogenic diet to all participants outpatient for a few reasons. First, study power will be increased for the key portion of the study: feasibility of MCT-KD in the home. Second, regardless of inpatient randomization group, all participants will have opportunity to try the

ketogenic diet as possible therapy, which should improve study recruitment and limit possible "nocebo" effect that an outpatient control group might otherwise be subject to.

Herein is proposed an inpatient pilot study of MCT ketogenic diet (MCT-KD) compared to standard American diet (SAD) in randomized, double-blind participant groups followed by outpatient study of a similar ketogenic diet with education and meal preparation guidelines, monitored for the inpatient portion with fingerstick beta-OHB levels, continuous glucose monitoring, and respiratory quotient and outpatient via online meal planning app, Diet tracking, correlating with motor physiology, cognitive inhibition and short-term recall, and EEG characteristics to establish which, if any, aspects of known PD test impairments may be modulated by ketogenic intervention in relation to suspected or likely metabolic pathways, to inform subsequent study into symptomatic benefit and/or disease modification.

4.3 JUSTIFICATION FOR DOSE

MCT ketogenic diet has been shown to be non-toxic (Traul 2000) and well tolerated at a dose of 30mL three times daily (Harvey et al 2018). This dose led to a mean increase in beta-OHB of 0.8mM by day 7 and shorter latency of keto-induction (more participants achieving level of 0.5mM by days 1-2), as well as lower mean glucose values, with mean beta OHB in the MCT oil group >1mM by day 6. Due to the reported higher incidence of GI side effects in that study, and expected lower GI motility due to autonomic dysfunction, a more moderate dose of 20% calculated daily resting energy expenditure or approximately 45-60 mL daily will be used. A similar dose of 40mL once daily in a cohort of AD participants the morning fasted state showed increase of beta-OHB by approximately 0.5mM at peak-dose, 90 minutes later (Reger et al, 2004). MCT as a supplement to ketogenic diet has also been studied in pediatric epilepsy and found to be useful to achieve a therapeutic effect in that population with a wider range of carbohydrate intake (reviewed in Liu et al, 2008). A similar use in PD might be established, and such determination would require further study such as proposed in this study. MCT permits a manipulation of ketogenesis between a ketogenic and non-ketogenic group while maintaining a similar diet composition, including same levels of protein, which is an important potential confound through its reported effect on L-DOPA intestinal absorption neutral large amino acid transporter.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Must be able to speak English
2. Able and willing to provide informed consent
3. Male or female older than age 50 years
4. Clinically probable diagnosis of Parkinson's Disease by UK Brain Bank Criteria, of moderate severity (Hoehn & Yahr Stage 2-4), with ability to safely walk independently for at least a short distance (20 feet) as determined on screening visit
5. BMI > 18.5, to minimize potential risk from expected mild weight loss from ketogenic diet
6. eGFR > 60 by MDRD equation (established on screening visit serum chemistry)
7. MOCA > 20, as well as having in the investigators' assessment the ability and willingness to adhere to either of the study diets
8. Agreement to adhere to Lifestyle Considerations (see section **5.4**) throughout study duration
9. Adhering to Usual Diet (SAD) at baseline, as per investigator determination

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Atypical Parkinsonism or symptoms suggestive of a diagnosis other than PD by clinical criteria
2. Family history of early onset PD (<age 40) or known personal genetically causal etiology of PD (e.g. SNCA duplication, Parkin, PINK, DJ1) by previously obtained genetic testing
3. Currently pregnant
4. Sarcopenia defined as low BMI (<22 Bahat et al, 2019) with clinically defined weakness
5. Medical history of cardiac arrhythmia, heart failure, stroke / cerebral hemorrhage, epilepsy, other disease of the central nervous system, active cancer, end-stage liver disease, advanced kidney disease (CKD stage 3 or ESRD), beta thalassemia, or any other medical condition deemed by the PI to pose an increased risk for taking part in the study.
6. Inherited or other metabolic disease known to be worsened by ketogenic diet, e.g. inherited defect of lipid or amino acid metabolism
7. Diabetes on SGLT2 inhibitor or uncontrolled diabetes, defined as Hemoglobin A1c > 8.0% on screening test
8. History of kidney stones or gallbladder surgery
9. Biliary / liver disease, defined on screening labs, by presence of any of the following: Total bilirubin (TB) > 2x ULN or > 2 mg/dL; AST >3x ULN; or ALT >5x ULN
10. Uncontrolled hypertension, defined as SBP > 180 mmHg or DBP > 105 mmHg on screening visit
11. Hyperlipidemia, defined by LDL >/= 160 mg/dL, as per ATP-III guidelines
12. Medical / psychiatric condition identified via clinical assessment in screening visit felt to impede completion of the study*
13. Presence of PD Psychosis or dementia, or other neuropsychiatric or psychiatric illness impeding consent and fidelity to the study intervention and/or measurements
14. Dietary or allergy restrictions as determined by research team to be prohibitive for the study
15. Inability to communicate and provide informed consent in English
16. No history of previous use of ketogenic or similar diet to a degree that could interfere with study blinding

*A thorough medical and social history will be performed during the screening visit including questions regarding alcohol and substance abuse. If active alcohol abuse or other current substance abuse is identified which could increase the risk of study participation, then participants will be excluded.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

N/A

5.4 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Participants who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches) will not be permitted while they are in the clinical unit.

- Abstain from strenuous exercise for 4 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (e.g., watching television, reading).

5.5 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).
Individuals who do not meet the criteria for participation in this trial (screen failure) because of a significant systemic comorbidity, concern of potential atypical or secondary parkinsonism, or PD psychosis or dementia may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

Community engagement was obtained by involving People with Parkinson's (PwP) who either adhere to ketogenic diet or who are interested in modifying their diet for PD or general nutritional health. These six individuals were identified through NIH PD Clinic as attendees of the clinic and initiated discussion about ketogenic, Paleolithic, otherwise low-carbohydrate diet or requesting dietary recommendations. With all of these individuals, the following was asked:

-what do you think about this study concept – comments, questions, concerns?
-what do you think about ketogenic diet? (if you have a sense about it at all)
-have you received dietary recommendations regarding PD or your general health, if so would you be willing to share what the gist of those are?
-do you consider your diet to have any particular impact on your symptoms, if so how? (excessive protein is well established to worsen PD symptoms in those who have more advanced disease with fluctuations, excessive carbohydrate not as clear, although metabolic syndrome is a clear risk factor for PD).
-do you think a one-week study with non-invasive testing aside from blood draws is reasonable without monetary compensation aside from travel expenses, on the basis of the potential therapeutic value of dietary modification and learning about its effects on your symptoms?

Initial feedback (n=25, predominantly via PD Facebook interest groups and also email) has been that ketogenic diet has been attempted by few of these individuals, and dietary recommendations are not standard in medical care for PD. There is agreement that both excess carb and protein can perturb symptoms of fatigue, tremor, bradykinesia, and so forth. There is some expressed concern from a few individuals that a one-week inpatient study with a standard diet vs. ketogenic diet might have difficulty garnering recruitment, with limitation in exercise regimen raised as a potential barrier. It will be important to distribute questions about the current study concept and dietary interest / engagement to a wider sampling of the community, which will be targeted via in-person discussion at local support groups, and continue through online media such as Facebook support groups. Additionally, one of the associate investigators is a PwP who has adhered to ketogenic diet for ten years and has provided valuable insight on the diet's anecdotal long-term effects on symptoms, tolerability and ADLs, guiding this study design.

Recruitment will include contacting PD patients who have been previously seen at NIH under protocol 01-N-0206, 93-N-0202, or 14-N-0086. The informed consent documents associated with the protocols

contain language that permits subjects to be recontacted for future studies and will be contacted via email, telephone or a mailed letter.

Recruitment will also occur through listing on ClinicalTrials.gov direct presentation) at local support groups.

Accrual will occur up to 2 screening visits / admissions per week, and expected average of less than that, perhaps 1-2 per 2 weeks.

There is one study site, NIH Clinical Center due to the specific inpatient study design. There will be no specific effort aimed at recruiting internationally. This is because the goal is to show feasibility and time series in a small-power pilot study, addressing mechanistic questions prior to any larger study that could show external validity in different disease populations.

5.6.1 Costs

The main cost to participants would be in local transportation across two study visits, defined as <(50) miles from the NIH Clinical Center. Travel expenses could be incurred in traveling for longer distances but would be eligible for reimbursement. Additionally, some additional cost may be associated with the two week outpatient ketogenic diet above usual food expenses, but given that MCT oil (approximately 25% of the daily fat) will be supplied, this is not expected to be a major cost and meal plans will be designed with cost in mind. The inpatient intervention and all testing would be provided to the participants free of charge. There is the potential for acute decompensation in vital function from relatively frequent causes - infection, cardiac arrhythmia, or thromboembolic event - or other cause, for which emergent acute care would be provided by Clinical Center without cost to patient, and transfer to nearby, affiliated or other medical facility could be performed at cost to the patient for services (e.g. tPA for stroke) not available at the Clinical Center. Due to the short duration of the study, the possibility of costs for emergency medical care is relatively low.

5.6.2 Compensation

For travel expenses to/from Screening Visit (#1) and/or Admission Visit (#2) and/or Outpatient Final Visit (#3):

- Reimbursement for Lodging if travel >50 miles: as per scale used for 01-N-0206 protocol
- Reimbursement for travel < 50 miles as per scale used for 01-N-0206 protocol
- Compensation will not be provided

6. STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

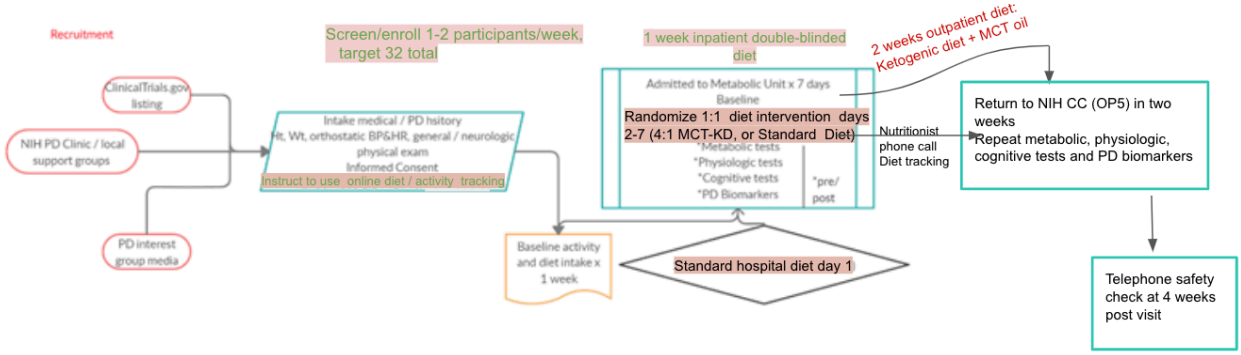
6.1.1 Study Intervention Description

Study Type and Design

Patients will undergo age and TUG time 2-factor stratified randomization to MCT-KD or MD groups by biostatistician who will keep the randomization scheme (see 9.2, Sample Size Determination). Randomization will not factor PD medications or DBS status, because there is no clear data to suggest

that certain medications, medication dosages, or DBS status will interact with dietary composition, either ketogenic or standard at standard protein intake. Study raters will remain blinded to diet group, because randomization scheme will be kept only by biostatistician and intervention status will be known otherwise only by nutrition staff, none of whom are involved in study rating. A possible exception is unblinding due to serious adverse event determined by study PI (non-rater) in consultation with safety monitor in which participant continues in the study, in which case data will be demarcated and not included in statistical analysis. Prior to admission, participants will be instructed to complete a log for 1 week of activities and diet at home. Upon admission to the Metabolic Unit and during hospital day 1, they will continue a regular, healthy hospital diet (carbohydrate predominant) and undergo baseline testing in mobility, physiologic and cognitive measures as per Section 3, Objectives and Endpoints). On hospital days 2-7, they will begin one of two diets: diet A, MCT-KD, according to set macronutrient ratios by daily energy: lipid 80% (25% of which is MCT oil), carb 5-10%, protein 10-15%, expected to be better tolerated than traditional NK, vs. diet B, standard American diet, lipid 30-35%, carb 55%, protein 10-15% . The goal is study time series of betaOHB, respiratory quotient, and continuous glucose ketosis measurements with tests of symptomatic impairment, with the hypothesis that nutritional ketosis augments mitochondrial function within hours of keto-induction and provides further benefit over days due to better functional neurotransmitter supply to key motor and cognitive synapses, aside from any long-term disease modification. Added benefits of an inpatient trial include monitored adherence and a look at biomarkers such as respiratory quotient in the metabolic chamber (CO2:O2 expected to go down due to diet), as well as easier access to daily repeated testing such as cognitive focused testing on executive function, memory and learning, as well as quantitative motor assessment of bradykinesia (keyboard, 9-hole pegboard), blinded UPDRS, and pain / fatigue assessment by subjective report.

Overview of Trial Flow and Events. Figure 1 highlights MCT-KD trial flow and events. We propose a screening enrollment of 1-2 participants per week for total of 32 participants. Upon meeting study criteria and consent, participants are asked to provide one week baseline diet and activity/exercise intake, and then be admitted to NIH CC for a one week study intervention assessing ketosis and symptom outcomes, safety monitoring and potential biomarkers of the diet on Parkinson's EEG and BDNF levels. During admission and discharge, communication will be maintained with participants regarding the requested outpatient portion, in which participants are provided meal plans to continue (or in the case of standard diet participants, begin) a standard ketogenic diet, with MCT oil in the home. The outpatient diet will be given as recommended meals and instructions to be reachable via telephone for twice weekly unscheduled calls from NIH CC Nutrition staff to populate diet adherence using diet tracking linked to study investigator's account. Additionally, study nutritionist / investigator (not involved in study rating) will perform unscheduled twice weekly phone calls to obtain post-screening baseline diet (one week) and outpatient KD intervention adherence assessment (two weeks). Participants will then be asked to return for a follow-up visit in NIH CC OP5 two weeks after discharge date. If the appointment date varies from two weeks for any reason, the same visit and measurements will occur, but visits will be demarcated separately for statistical analysis.



Screening procedure: Following recruitment, potential subjects will be pre-screened by telephone, in person, or via email communication according to the inclusion / exclusion criteria, discussion of the scope of the study and study design, expected potential benefits and side effects. If the potential candidate meets the study eligibility criteria, a screening visit will be scheduled at NIH CC outpatient clinic. The estimated duration of the screening visit is 4 hours, and may be spread over 2 seperate days if necessary. Upon registration, an order will be submitted for metabolic tracking (PCO2:PO2 gas exchange) for calculation of daily resting energy expenditure.

Informed consent will be obtained prior to any tests or procedures being conducted at the screening visit.

During the screening visit, history, physical exam, focused neurologic exam, Unified Parkinson's Disease Rating Scale (UPDRS), Geriatric Depression Scale (GDS), Non-Motor Severity Scale (NMSS), blood draw consisting of 1 gel-barrier tube (gold, red, or mottled red-gray) for BMP, HCG for women of childbearing potential), and Timed Up and Go test, as well as randomization by study biostatistician. Results of study group allocation will be conveyed by biostatistician to study dietician to prepare for inpatient diet formulation.

<u>Active Intervention: The Ketogenic Diet.</u> The typical American diet contains ~50% carbohydrate, 35% fat, and 15% protein calories. The proposed KD proposes decreased carbohydrate (5-10%), greatly increases fat (80%), which becomes the primary energy source, and leaves protein unchanged (15%) calories. The ratio of lipid (fat) to non-lipid content (carbohydrate and protein) defines different NK plans. Unlike "Western" high fat diets that do not restrict carbohydrates, NKs do not significantly increase or may decrease triglycerides, insulin resistance, and body weight.

MCT oil consumption can increase overall fat intake, enhance ketosis, and allow more absolute carbohydrate consumption. Furthermore, in the outpatient part of the study, complex carbohydrates will be given through diverse vegetables including non-starchy green leafy vegetables and tomatoes. The lipid ratio will be MCT: other lipids at a 1:3. Multiple trials of AD patients (e.g. Reger et al 2004, Ota et al 2019) demonstrated this approach is feasible for AD patients, and thus toleration is not considered to be of significant concern. MCTs, though, can cause gastrointestinal discomfort, and this will be monitored. In the Reger et al and Henderson et al studies cited, an emulsified MCT formulation and gradual titration schedule minimized this complaint.

Blood fingerstick ketone concentrations quantify depth of ketosis. Adherence will be ensured through clinical provider/nurse check-ins and meal preparation in coordination with dieticians and Clinical Center nutrition staff. We will determine fingerstick β-hydroxybutyrate levels at baseline and at two daily defined intervals from meals and medications (once daily on day 7). Ancillary proxy testing of ketosis will additionally occur via continuous glucose monitoring (Freestyle Libre Pro System sensor, Abbott, FDA approved for healthcare professional use with diabetes, for non-FDA approved research use in this study, to gauge indirectly ketosis value in continuous fashion).

All participants will receive dietary counseling and educational material from study dietician/nutritionist. Counseling will include planning outpatient meals that are ketogenic incorporating MCT oil, with the goal of prioritizing accessibility in grocery stores for participants to obtain relatively easily; relatively lower cost ketogenic foods; and considering nutritional value.

Because the intended use of MCT oil is to facilitate a ketogenesis state along with diet and nutrition to mitigate symptoms of Parkinson's disease, MCT oil may be considered to be a drug for the purpose of the study. The use of MCT oil is not intended to support a new indication for use or any other significant change to the labeling; the drugs are already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

MCT oil will be used as a means to facilitate achieving ketogenesis in a manner that is expected to be more feasible for patients than the ketogenic diet alone. MCT oil is commercially available and meets GRAS FDA exemption (**see protocol 2.3.1a**). While MCT oil is novel in the study population and has not yet been studied in patients with Parkinson's disease, the expectation is that adverse events to be similar to those reported in several studies of patients with Alzheimer's disease. The oil is not being modified in any way and will be used according to approved parameters as a dietary supplement to achieve a ketogenic state, which is the primary goal of this study.

An IND exemption was filed with the FDA, and the final outcome was FDA determination that the study does not appear to raise safety concerns and accordingly, the research study is not expected to comply with the IND reporting requirements for this study. The use of MCT oil in this research study will be within the parameters and standards of the GRAS FDA exemption for this product.

6.1.2 Dosing and Administration

Dose of the Liquigen® (KetoCal) MCT oil will be calculated individually based on 20% participant daily energy expenditure using the Weir formula. Meals will be combination of solid and blended liquid with care taken for blinding study subjects to ketogenic or standard diet composition.

6.1.2.1 Drug Administration

The MCT oilLiquigen® (KetoCal will be incorporated into food / beverages for participants in the MCT-KD group by NIH CC Nutrition Department staff, who will not disclose group identity to study raters. Record of food and beverage consumption will be maintained using weight and data entry as per usual practice in nutritional studies at the NIH CC. The purpose of the notification will be to ascertain the

degree of missing data for ketosis intervention; determine if any quality control might be helpful; and see if additional ketosis data are required.

The standard and ketogenic diets will be provided by the Nutrition Department of the Clinical Center. Both diets will be as per provided menu in collaboration with Metabolic Unit staff (study dietician and others). Standard diet will adhere to macronutrient composition lipid 35%, protein 10-15%, carbohydrate 50-55%. Ketogenic diet will incorporate restriction of carbohydrates to reach 80/5-10/10-15 (lipid:carb:protein daily energy) values. The total daily energy will be calculated from respiratory gas exchange in the Metabolic Chamber using the Weir formula (Weir, 1949): Metabolic rate (kcal per day) = 1.44 (3.94 VO_2 + 1.11 VCO_2). Both ketogenic and standard diets will be eucaloric, providing recommended daily calories.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

N.A.

6.2.1 Acquisition and Accountability

The Liquigen® (KetoCal)MCT oil is carried by the Clinical Center Nutrition Department and will be made available for the diet preparation.

6.2.2 Formulation, Appearance, Packaging, and Labeling

As per Liquigen® (KetoCal) Product Bulletin
(URL: < https://shop.myketocal.com/product/liquigen>):

- **Ingredients:** Refined coconut and/or palm kernel oil, water, citric acid esters of mono and diglycerides (emulsifier), mono and diglycerides (emulsifier), citric acid.
  - UnflavoredFor oral or enteral use
  - kcal/mL: 4.5
  - Caloric Distribution (% of kcal)
    - Protein: 0%
    - Carbohydrate: 0%
    - Fat: 100%

Recommended Usage:
- Oral Administration: It may be mixed into beverages and foods.

6.2.3 Product Storage and Stability

This product will be stored in the original plastic 250 mL bottle.

6.2.4 Preparation

MCT Oil® (Nestle) MCT oil will be mixed by Nutrition Department Dietary services at the Clinical Center. It will be added to other ketogenic foods prepared as per the study menu.

Outpatient ketogenic diet

According to participant decision after discussion during the screening and admission, willing participants are instructed to continue ketogenic diet as defined by CC nutrition staff customized from provided meal plans provided to each participant.

Diet tracking with information on macronutrient and micronutrient composition will occur via unscheduled phone calls by study dieticians conducting 24 hour food recall, input to NDSR04 software.

6.3 Measures to Minimize Bias: Randomization and Blinding

As described in Section 9.2 (refer for details), study participants will be randomized after screening visit by study biostatistician. The intervention allocation will be withheld from all study investigators and raters until after the conclusion of all measurements, who will remain blinded to participant allocation for the duration of the study. Refer to Section 9 for details of randomization.

The study nutrition staff will remain unblinded in establishing participant-individualized meal plans. S/he will not disclose any diet details of the admitted participants and will not participate in any of the study measurements. Other investigators will be blinded except due to adverse event or inadvertent event.

Nutrition staff (CC Nutrition Department) will design the blinded diets according to the macronutrient ratios and MCT oil supplementation specified in the study (from calculated daily total energy expenditure by gas exchange on screening visit using Wier formula). Aside from the addition of MCT oil comprising 25% of daily lipids, the diet will be similar to what was used in existing study at the NIH CC Metabolic Unit, NCT03255031 Ketogenic Diet in Alcoholism, as per clinicaltrials.gov description:

Dietary Supplement: Ketogenic Diet (KD) / Standard American (SA) Snacks and Shakes

"Ketogenic Diet (KD) / Standard American (SA) Meals and Shakes: Participants will be randomized to a meal plan of either a ketogenic diet (KD) or standard American (SA) diet at the time they sign the consent. For each meal at breakfast, lunch and dinner, the diets will consist of SA meal (carbohydrate rich) or KD meal depending on treatment randomization... [ ]...To ensure that the diet is double blind...[ ]...solid snacks are always ketogenic and shakes are either KD or SA."

From conversation with NIAAA study PI and study nutrition staff, when participants were asked about which diet they believed they received, they usually thought they were receiving ketogenic diet, which appears to support the integrity of the dietary blind.

Based on the literature of ketogenic diet pilot studies (of 3 months duration or less) that no major adverse event was reported in three ketogenic studies in PD and another three in AD, it is unlikely that a serious adverse event (SAE) related to the intervention will occur. Monitoring will occur by daily clinical assessment (AI / metabolic unit staff) and electrolyte testing.

If there is a major adverse event, including but not limited to: Na <135, K < 3.4, Mag <1.0, HCO3 < 22, and/or vomiting, study investigators including PI will be immediately made aware by Metabolic Unit staff, discussion will occur with the Independent Medical Monitor within 24 hours as to recommendations for therapy, and participant will be withdrawn from the study and study investigators unblinded to the participant's intervention status unless there is consensus by the IMM and Metabolic Unit staff that ketogenic diet can be continued with other measures taken (e.g. fluid rehydration, anti-nausea medication) or participation continued on a modified / less stringent ketogenic diet.

Despite double-blind design, unblinding may occur inadvertently. The participants will be instructed beforehand to make no mention of their diet, same as Metabolic Unit nurses and dieticians. If however unblinding of study rater(s) does unexpectedly occur, participants will by default remain in the study but be discounted from study power calculation and marked separately for statistical analyses.

6.4 STUDY INTERVENTION COMPLIANCE

Study adherence will be assessed primarily through blood and respiratory ketosis values, both directly via beta-OHB phlebotomy at defined intervals (three times daily) and indirectly via POC glucose checks, continuous glucose monitors, and daily respiratory quotient (pCO2/pO2, expected to decrease from approximately 1.0 towards 0.7 as a function of predominantly glucose to predominantly fatty acid metabolism). Adherence will be assessed also through records of diet consumption made by Nutrition Department staff (AI) using instructions to consume 100% of provided meals and weighing meals before/after consumption, logging data using ProNutra software. All willing study participants will be counseled on ketogenic diet in preparation for the outpatient portion of the study unless they have communicated that they will decline the outpatient study. They will receive a schedule of possible meals to select from for the two weeks of outpatient study and to establish a means of contact with study nutritionists for adherence estimation. During these two weeks, study nutritionists will call them twice weekly at unscheduled times for 24-hour food recall review, and data from these calls will be used to determine outpatient adherence.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

No adjustments will be made to patient's home medication regimen including all Parkinson's Disease medications. Any omissions whether in error or patient choice will be recorded in CRIS.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from dietary intervention does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

Holding criteria: Study intervention(Liquigen® (KetoCal)) and ketogenic diet) will be withheld if clinically significant cardiac arrhythmia is detected on baseline ECG (please refer to Clinical Evaluations Section 8.2 for details).

The data to be collected at the time of study intervention discontinuation will include the following:

- diet menu and record
- patient interview with worksheets and symptoms
- height, weight, vital signs including orthostatic (supine x 10min, immediately and 3min after standing); physical examination with focused neurologic examination

Stopping criteria:

1.) Patient request
2.) If any investigator judges that continuing the procedure would be detrimental to the patient's health and well-being or that clinically meaningful data is not being obtained for other reasons.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Completion of study intervention
- Disease progression which requires discontinuation of the study intervention
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Investigator discretion
- Positive pregnancy test
- Participant unable to receive study intervention for more than 1 day

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Prior to removal from study, effort must be made to contact all study participants via telephone to determine safety regarding the intervention approximately 2-4 weeks following the last dose of study therapy.
Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If the participant meets an exclusion criterion (newly developed) that precludes further study participation
- Subject has completed the study follow-up period

- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for the admission following the screening visit and is unable to be contacted by the study site staff.
The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit over the course of the following one week and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

Recruitment will occur from existing cohort of PD patients that have enrolled in NIH protocols 01-N-0206, 93-N-0202, and14-N-0086. All informed consent documents associated to the referenced protocols contains language that obtains permission from subjects for their information to be saved and to be re-contacted by study investigators for future research studies at the NIH. There will be access to prior records in CRIS, paper chart, and genetic testing. The data are accessible by associate investigators of all the above protocols. All federal and state laws as well as requirements of the NIH will be followed regarding patients' PHI.
.
Participants either previously seen under the protocols listed above, or otherwise contacted or who contact study investigators with interest in the study will be pre-screened with the request for /in most cases

obtaining outside documentation of Parkinson's Disease clinical care, with exception being made only if the records are not available upon request, however a thorough attempt will be made at verification of existing diagnosis. These outside documents will be requested of participants to be faxed to the Study Investigator (PI), or permission be granted for PI to request this from the participant's physician office directly. For participants pre-screened who have participated in 01-N-0206, prior documentation by the protocol investigators will likely be considered to be sufficient without outside documentation.

At the screening visit after participant's informed consent is obtained, data will also be obtained from patient self-report, however effort will be made to verify this with physician or ancillary medical care documentation.

8.2 EFFICACY ASSESSMENTS

As one of the study aims is to determine feasibility of MCT supplemented ketogenic diet, the efficacy of primary objective of study feasibility as per recruitment, retention, acceptability, and adherence will be determined by a composite outcome, in which all criteria are met: completion rate of both inpatient 1 week and outpatient 2 week segments, with target of >80% for each; recruitment target of 32 participants reached; adherence to outpatient diet with mean daily carb </= 10%; intervention is acceptable to all participants by Likert scale >/= 2/4 of likelihood of using this therapy in the future and effectiveness in symptom reduction.

Efficacy of secondary objective of TUG reduction will be assessed by between-group mean difference at 7 days (MCT-KD vs. SAD). It is presumed that the standard (control) diet will not provide any issues of feasibility. Refer to Section 4.1 Study Design for direct and indirect determinations of ketosis.

Timed Up & Go (TUG)
Efficacy of TUG will also factor effect of time, via repeated measures ANOVA for TUG that will be tested three times per session and fastest time used, with same timing relation to dopaminergic medications.
Efficacy will be tested further in the between group difference between the two diets on outcomes of the following, again with same timing regarding dopaminergic medications:

Exploratory measures will be done as per test schedule in Section 4.1, generally once daily or less.

Stroop Word-Color Interference
this test has been shown to be impaired in PD as part of the executive network of actively choosing the stimulus while inhibiting the background. The latency will be determined from a computer test.

N-Back Task (% correct)
This test (Kirchner, 1958) involves a series of visual stimuli such as letters, and then participants are asked if each stimulus matches a stimulus n trials before. Performance is shown to be impaired in PD to a greater degree than in normal aging

Simple Reaction Time (SRT)
SRT (same cue on computer screen, press button; machine is used already for Chronic Fatigue Syndrome protocol studies and will be made available for the study). PD is consistently shown to have increased latency that is attributed to the akinesia as cardinal motor symptom. The increase in latency is reduced by

Levodopa therapy (Muller 2001, 23729617), relatively unaffected by repetition / motor learning, and is hypothesized also to improve with metabolic ketosis.

Keyboard MN and PQ speed and rhythm analysis
The Keyboard test is used already for the SCA7 protocol by Human Motor Control neurophysiologist and will be made available for the study. Rhythm can be obtained from the raw data, which imputes the time of the keystroke into a text document and can be analyzed in frequency vs. speed analysis. PD bradykinesia involves a sequence effect of both reduction in amplitude and speed of repetitive fine motor movements that is well established. Therefore, as speed decreases there is expected greater variability in frequency).

9-hole pegboard test
This standardized, quantitative test of finger dexterity has been studied in Parkinson's Disease (e.g. Earthart 2011) and found to correlate with Hoehn & Yahr disease severity with times of 20s for stage I ranging to 40s for stage IV. It can be done with a single investigator, stopwatch on a table, and the equipment is already available (being used for Neuro testing in 7SW for SCA7).

8.2.1 Clinical Evaluations

Physical examination
Participants will be examined at screening visit by board certified neurologist and movement disorder specialist with focused examination:
general, cardiopulmonary auscultation, neurologic exam with focus on motor or other system disorders (such as sensory, musculoskeletal) related to Parkinson's. There will also be UPDRS and request for video recording. Video record would require separate signed informed consent as per NIH protocol. Video recording is done for research purpose only (quality assessment by averaging scores of in-person rater with a separately blinded video rater).
Examination with requested video recording as per above will be repeated on day of discharge (post-intervention). Examination as per above will also be repeated on day of admission (pre-intervention), without request for video recording unless a significant clinical finding is observed in difference from the screening visit.

Vital signs and Heart rate variability
Vital signs will be done as per routine care in the Metabolic Unit and will also include daily orthostatic check to be done by Metabolic Unit staff or credentialed study investigator. Daily heart rate variability will be performed with pre/post intervention (daily days 1-7 and at week 3) heart rate responses to a) deep breathing at 6 cycles per minute (5 cycles E:I ratio) and difference between average max and min heart rates; and b) Valsalva Ratio (HR max : HR baseline) with Valsalva Maneuver x 15s at 30-40 mmHg (days 1, 7, and week 3).

Radiographic or other imaging assessments
ECG – ECG will be performed at screening or Day 1 of admission (depending on availability), however must be performed prior to diet intervention. A routine ECG will be performed for confirmation of normal cardiopulmonary function. If arrhythmia is detected, Cardiology will be consulted and incorporating their recommendations, decision about remaining or withdrawing from the study based upon the underlying etiology sinus (more likely stay in the study) vs. non-sinus rhythm aberrancy (more likely withdraw from study) as per inclusion/exclusion criteria.
EEG - regional changes in spectral power (theta, alpha, beta) and nonlinear corticocortical synchronization measure via phase lag index (focusing on frontotemporal, parietotemporaloccipital areas

in alpha1 and alpha2 frequencies, and reduction in theta power in temporal-central regions) as per Chaturvedi et al, 2019.
EEG will be performed pre and post intervention in 7SW Neuro Testing area for a period of 20-30 minutes (1 hour session), under the supervision of a neurophysiologist and/or a neurologist trained in the technique (AI).

8.2.2 Biological specimen collection and laboratory evaluations.

Blood tests will be drawn twice daily for plasma beta-OHB levels and plasma dopamine through contracted vendor(s), to be determined based on competitive bid process and stored at -4 C as per recommended processing/batching. Blood draws will also occur on screening visit, hospital days 1 and 7 of admission, and week 3 visit for metabolic tests at a CLIA certified laboratory (NIH Clinical Center Laboratory) as per section 8.2.2 below.
Blood draws: 1 x (screening) + twice daily (admission days 1-6) + 1x (admission day 7) + 1x (week 3 final visit)

Study total: - volume of blood: 38 small tubes (1-2 mL each), approximately 40-80 mL
- blood draws: 15

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

Special assays or procedures required:
Continuous glucose monitoring (Freestyle Libre Pro System sensor, Abbott)
The monitor will be provided to participants upon enrolling at the screening visit (#1) to obtain one week of baseline data on usual diet, and continued for the duration of the admission. Two weeks of data are provided in the system memory. If there is a delay between the screening visit and the admission, participants will be instructed to stop the monitor at one week in order to preserve the second week memory storage for the admission.

Venous beta-OHB level and dopamine level
Blood draws will be performed at defined intervals twice daily - at 7am, and at 2 hours after dinner (8pm). Timing of the blood draws is regarded as important to be as accurate as possible. Plasma dopamine will be tested on inpatient days 1-3, and plasma BOHB will be tested on inpatient days 1-7 and at week 3. The testing and reporting will be done in accordance with CLIA-certified NIH Clinical Center Core Laboratory.

Respiratory exchange ratio (quotient), pCO2:pO2
Will be performed on the Metabolic Cart at screening and week 3 outpatient clinic visits as per previously described method (Hall 2015, 26278052)

Metabolic labs (blood): Hgb A1c, lipid panel, insulin, glucose, cortisol, TSH, T3, free T4,, BDNF
Will be drawn day 1, day 7, and week 3 (final study visit), except for HgbA1C (screening, week 3) and lipid panel (screening, day 7, week 3)
Non-heparinized sterile tube will be used for transport / storage in the NIH Clinical Center Core Laboratory.
The testing and reporting will be done in accordance with CLIA-certified NIH Clinical Center Core Laboratory.

Electrolytes (BMP, Magnesium) will be checked daily on inpatient days 2-6. If there is abnormality possibly or probably attributed to the intervention or determined to be medically significant by study

investigators, it will be repeated on study days 6 and 7 and appropriate additional steps taken (e.g. hydration, anti-nausea medication, decreasing the lipid or MCT ratio of the diet, and possibly additionally including blood gas monitoring / transfer to ICU) in coordination with metabolic unit staff and the independent medical monitor.

Daily survey: Parkinson symptoms, ketoinduction symptoms and gastrointestinal motility

Two surveys will be administered daily, one detailing common Parkinson-related motor and non-motor symptoms (drawn from PI / AI clinical experience) as well as typical keto-induction symptoms (adapted from Harvey et al, 2018) and the other survey detailing gastrointestinal motility, adapted from IFFGD, URL: https://www.iffgd.org/publications-library/library/341.html

Blood and urine samples will be discarded as per routine policy.

8.2.4 Samples for Genetic/Genomic Analysis

PD inherited genes and family/linkage studies will be reviewed when available (prior enrollment in 01-N-0206 or other neurogenetic study). There will be no samples collected for genetic analysis under this protocol.

8.2.4.1 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Participants will be provided a study number, and storage/reporting will be only via this number. Study number will be linked to PHI only via a document maintained by unblinded NIH staff investigator not involved with study measurements (biostatistician) that will be removed from other investigators except for instances of unblinding due to adverse events or study withdrawal.
Measures of securing participants' PHI will be maintained as follows:

- Generation of study number by unblinded investigator (AI) in place of PHI for records of independent / dependent measures
- No PHI in daily measurement records (study number and recorded values, including daily self-report worksheets)
- All records maintained in locked cabinet or on NIH Secure server
- PHI for purpose of clinical care provided during admission maintained on password protected CRIS electronic medical record
- Data sharing with other researchers such as NINDS supervisors, co-workers, or Grand Rounds will be in de-identified fashion, except where Video Consent was obtained with provision of removing facial / identifying features was provided to participant but declined
- Data will not leave NIH except in the format of publication or presentation at professional meeting as per Data Sharing bullet item above

8.2.4.2 Management of Results

Results will be provided to participants in two formats: a discharge summary and a debriefing on the day of discharge or as otherwise determined with the participant. The discharge (DC) summary will include all laboratory results, including lipid profile, A1c, insulin, that in combination with blood pressure/height/weight/BMI and body fat % (bioelectric impedance) are significant to the metabolic and cardiovascular health, e.g. criteria for metabolic syndrome, HOMA-IR (insulin resistance calculation), and Framingham Risk score. DC summary will also include results of clinical assessments (e.g. Parkinson motor and non-motor symptoms / phenotypes / severity) and validated rating scales such as UPDRS, MOCA, and NMSS. Results

will not include ketosis values, EEG results, and cognitive / physiology / autonomic measures obtained for research purposes that lack current health implication. The debriefing will review DC summary results as well as give opportunity for researcher (PI / Metabolic Unit staff) to present clinical synopsis and share aspects of the participant's research data as per expressed interest, as well as to provide education on diet given, general dietary recommendations, and summary of the intervention and research overview. Participants will be invited to review any publications and will be contacted for permission prior to presentation of any video.

The NIH Clinical Center Laboratory is CLIA certified, CLIA ID Number - 21D0665373

8.2.4.3 Genetic counseling - Not applicable

8.3 SAFETY AND OTHER ASSESSMENTS

- Biological specimen collection and laboratory evaluations.
  For metabolic analysis, blood samples will be drawn at screening visit (basic metabolic panel), baseline (admission pre-intervention) and at discharge (admission post-intervention), as per Section. Additionally, blood will be drawn for beta-OHB levels at defined intervals (see Section 1.3 Schedule of Activities for details)
- Special assays or procedures required - continuous glucose monitoring

- No storage of specimen required. The data will be downloaded from the sensor attached to skin of the back of the upper arm to the Reader twice, on admission and on discharge.
- Heart rate variability monitor
- No storage of specimen involved. Data will be stored on a central application

- Counseling procedures, including any dietary or activity considerations
  Counseling will occur at study debriefing visit on day of discharge or as otherwise arranged, and may also occur at any point during the one-week admission (Visit #2) or during the screening visit (#1). Counseling will be primarily focused on healthy diet, including low glycemic index, DASH diet, and Mediterranean diet as per USDA guidelines, and will emphasize that ketogenic diet remains a matter of research interest regarding previously found / putative benefits for Parkinson's Disease, however is congruent with aspects that are recommended by dieticians and physicians, such as minimizing salt and high glycemic index refined carbohydrates.

- Assessment of study intervention adherence - see Study Intervention Compliance, section 6.4
  Administration of questionnaires or other instruments
  At screening visit (#1), participant will be asked to fill out PDQ39, and UPDRS (Parts 1, 2 and 4) and NMSS will be administered by PI. On the day of discharge, UPDRS and NMSS will be repeated.

- Assessment of adverse events.

There will be daily monitoring performed by metabolic unit staff and communicated to PI if concern, in turn discussing with independent medical monitor (IMM) and possibly unblinding AI if additional care required such as stopping the intervention, transfer to ICU. There will be no modification to the diets as outlined, to facilitate maintaining blinding and primary and secondary objectives of feasibility and ketosis ascertainment, respectively. There will be weekly meetings by investigators if any adverse events occurred as per Policy 801 (Refer to Section 8.4 for descriptions).

Intervention side effects will be identified by the study LIP who is monitoring the patient during the admission and has daily contact with the participant. With blood glucose monitoring during the study, the nurses will contact study investigators for blood glucose value < 70 mg/dL. The hospital protocols are triggered for BG <40mg/dL that involve sending sample to NIH CC lab, including contacting study investigators for emergent care. For blood glucose 40-70, there will be the following procedure: if asymptomatic and fully alert, 1 cup juice / crackers, recheck in 30 minutes, repeat as necessary. If symptomatic (such as light-headedness, headache, nausea) / mental status lethargy, provide juice / crackers and contact study investigators for possible IV D50 administration.

The literature on safety of the ketogenic diet in adults in clinical trials is robust, including several trials in Parkinson's disease (Krikorian et al 2019, Phillips et al 2018, Vanitallie et al 2005) . The majority of these trials were conducted in the outpatient setting and adverse events were quite rare and typically minor in severity. Similarly, MCT powder/oil as a supplement to accelerate ketogenesis is reported to be safe in Alzheimer's Disease patients (Reger et al, 2004) as well as healthy volunteers (Harvey et al, 2018). Participants will be provided with contact information for the study team and asked to report any concerns, problems or new symptoms. During the 2-week outpatient phase, telephone monitoring may also occur as needed.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include cardiac arrhythmia requiring intensive treatment in an emergency room or at home, or pancreatitis / kidney stones that do not result in inpatient hospitalization.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

Serious adverse events associated with ketogenic diet are rare and reported predominantly in pediatric epilepsy population with different comorbidities and varying neurodevelopmental impairment, thus are not directly applicable to the adult population with Parkinson's disease. Potential risks associated with ketogenic diet include dehydration related to natriuresis, electrolyte depletion, metabolic acidosis, vomiting, and elevated cholesterol and lipoprotein levels. Adverse events associated with these risks are not defined in frequency. Three pilot studies of ketogenic diet in Parkinson's Disease did not report any serious adverse events.

As a preventative measure, PD patients with cardiovascular and other major comorbidities will be excluded from the study, and EKG will be performed at admission baseline. Daily electrolyte testing will occur in all participants on study days 2-5, and daily clinical assessment for volume status will occur by metabolic unit staff and AI with maintaining blinding as possible in the absence of adverse events. Reporting of events such as vomiting or other ketogenic diet induction symptoms will occur to metabolic unit staff and relayed to study PI (not involved in rating) and independent medical monitor (IMM), except when medically necessary for unblinding to occur immediately (i.e. to provide emergent medical care). Baseline and end of study lipid panel will be measured and results made available to participants, with medical counseling to be provided in debriefing session on further checking outpatient with primary care physician if elevated or showing an upward trend.

Relationship to Study Intervention

All adverse events (Aes) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.4.3.2 Expectedness

IMM will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

AE will be solicited through daily keto-induction symptom worksheets. Opportunity for unsolicited AE will be provided / encouraged, with instructions for conversation to occur initially with study PI / IMM to maintain blinding of study raters, when AE is not serious and is non-emergent.
Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.
Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study PI will communicate to IMM and record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. Study AI will also be involved in evaluation after study completion when non-emergent. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

Reportable events will be tracked and reported in compliance with Policy 801.

8.4.6 Serious Adverse Event Reporting

The study investigator will immediately report to the IRB any serious adverse event, whether or not considered study intervention related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the IRB.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC) and should be provided as soon as possible.

The Principal Investigator (PI) will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the PI's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the PI determines that the information qualifies for reporting.
Reportable events will be tracked and reported in compliance with Policy 801.

8.4.7 Events of Special Interest - N/A

8.4.8 Reporting of Pregnancy - N/A

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.5.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

1 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

Primary hypothesis:

Nutritional ketosis supplemented by MCT oil is feasible within a Parkinson Disease cohort as defined by an acceptable retention rate and outpatient adherence.

Primary outcome: Feasibility of nutritional ketosis(ketogenic diet with 80% lipid including MCT oil, 10-15% protein, 5-10% carbohydrate), in Parkinson Disease cohort defined as a composite outcome meeting all of the following criteria: recruitment target of 32 or as per interim power analysis defined in Section 9.4.6; retention rate > 80% at study end; adherence to ketogenic diet in the outpatient portion defined as mean carbohydrate energy intake <10%, and intervention is acceptable to all participants by Likert scale >/= 2/4 of likelihood of using this therapy in the future and effectiveness in symptom reduction, from all study participants at 3 weeks.

Secondary hypothesis:

Nutritional induced ketosis (NK) increases mobility based on TUG score in a Parkinson Disease cohort at 7 days compared to usual diet (35% lipid, 10-15% protein, 50-55% carbohydrate). Secondary outcome: timed Up & Go test (TUG score) at study day 7 (fastest of three trials)

H_0: $\mu_1 = \mu_2$, where μ_1 and μ_2 are the population means for the NK diet group and usual diet group respectively vs. H_1: $\mu_1 \neq \mu_2$.

Exploratory hypotheses:

Hypothesis A: Nutritional ketosis improves PD symptomatology in cognition (improved attention, recall, and executive function, and motor function akinesia and tremor within six days, as assessed by: Stroop word-color interference latency (executive function), n-back recall task (attention and working memory), simple reaction time (akinesia); fingertap speed and rhythm MN and PQ keyboard tasks (fine motor), and autonomic function (heart rate variability at rest and with deep breathing / Valsalva Maneuver tests)

Hypothesis B: The mechanism of symptomatic reduction of NK in PD is both augmented mitochondrial metabolism (direct, rapid) and augmented neurotransmitter and growth factor synthesis (indirect, delayed), especially GABA and dopamine, as assessed by resting state EEG spectral power, phase coherence, and M1 waveform normalization. Accordingly, effect sizes for exploratory outcomes will be greater at 3 weeks than 7 days.

Exploratory outcomes daily (days 1-7), week 3

ON/OFF time (daily subjective worksheets), keto-induction symptoms (daily worksheets or self-report), UPDRS-III motor score, MN and PQ keyboard tasks, simple and complex reaction time, 9-hole pegboard test, 3-back recall, Stroop word color interference and latency, tremor amplitude, heart rate variability heart rate variability (SDNN, pNN50, rMSSD, power spectral analysis).

Exploratory outcomes day 1, 7, and week 3:

Valsalva Maneuver and deep breathing cardiovagal tests), plasma BDNF, Geriatric Depression Scale (GDS), EEG sensorimotor spectral power (Hall et al, 2014) and M1 waveform peak:trough asymmetry (Voytek and Cole, 2017).

As an effect of the intervention at three weeks, there is expected to be decrease in EEG beta power on the more affected hemisphere clinically, relating to attenuation of pathologic pallidal GABA efferents to a greater extent than at 7 days or baseline. Similar trends are anticipated for attenuation of M1 waveform asymmetry, reduction in UPDRS total and subtotal scores, decreased latencies and total time of motor task performance tests, improved accuracy and speed of cognitive tasks, and increase in plasma BDNF and heart rate variability measures. The effect on depression symptoms is unclear but reduction is proposed.

9.2 SAMPLE SIZE DETERMINATION

Power analysis was performed based second outcome, TUG score, rather than feasibility outcome due to clinical significance of the former for both mobility and fall risk that is felt to be an important justification for this intervention as a pilot study, whereas feasibility ascertainment does not justify the study but merely indicates lack of existing clinical use of this ketogenic diet protocol.

Considering range of TUG tests reported in Parkinson's Disease off dopaminergic medication of 8-17s (Foreman 2011), mean of 10-15s with SD 2.15-3.7s (Foreman 2011, Brusse 2005, Dal Bello-Haas 2011), and association of 1s increase in TUG time with 5.4% increase in odds of reporting a fall (Nocera 2013), it is suggested that fall rate can be reduced by 20% with a 4s reduction in TUG time, which is therefore considered to be a clinically meaningful difference. Minimal detectable change is reported as 4.85s (Dal Bello-Haas 2011) and 3.5s (Huang 2011).

Based on these studies, the difference of 4.5s between the group means, which was considered as clinically important, and standard deviation (SD) of 4s (or effect size of 1.125) were used for power analysis. A two-sample t-test was applied to estimate sample size by assuming that the primary outcome, TUG, followed normal distributions, and the variances of the two groups were equal. Power analysis indicated that a total of 28 subjects, 14 per group, will be needed to achieve a power of 80% to detect the difference of 4.5s at significant level of 0.0492. The sample size calculation considered one planned interim analysis scheduled to be conducted when 8 subjects per group have completed. Using O'Brien-Fleming approach, the interim analysis will be conducted with bound, p=0.0054 (t-value=3.287, df=16) and final analysis with bound p=0.0492 (t-value=2.06, df=26). By anticipating 10% dropout rate, we will need to recruit a total of 32 subjects (16 per group).

Randomization
Stratified randomization method will be used to control the covariates of age (<=65 vs. >65) and TUG baseline score (>=11.5s, vs. <11.5s Nocera 2013). With these two covariates, there are 4 possible block combinations (TUG<=11.5s & age<=65, TUG<=11.5s & age>65, TUG>11.5s and age<=65, TUG>11.5s & age>65).

9.3 POPULATIONS FOR ANALYSES

32 subjects randomized to either ketosis diet or a low fat (standard American) diet

9.4 STATISTICAL ANALYSES

9.4.1 Analysis of the Primary Endpoint

Descriptive statistical analysis will be performed to assess the feasibility.

9.4.2 Analysis of Secondary Endpoint

To evaluate the diet effect on the TUG score, analysis of covariance (ANCOVA) will be performed with age and TUG score at day 1 as covariates, and TUG score on day 7 as a dependent variable.

Repeated measures ANCOVA with age as a covariate will also be performed to evaluate the within-subject effect: baseline day 1 vs. day 7 (inpatient) vs. 3 weeks (outpatient) of the intervention, and between subject-effect (diet): ketogenic diet vs. standard American diet.

9.4.3 Analysis of the Exploratory Outcomes

ANCOVA will be also used to examine the diet effect on the other exploratory outcomes, where each outcome measure over time will be used as a dependent variable with age and the outcome at day 1 as covariates.

Longitudinal analysis methods, such as repeated measures ANCOVA, random coefficient model or mixed model, will be used to examine the change in the exploratory outcomes over the six days**.** Within-subject

(mixed model) and across-subject (spearman or Pearson) correlation analysis will be applied to evaluate the relationship between outcome variables.

9.4.4 Safety Analyses

N/A

9.4.5 Baseline Descriptive Statistics

Descriptive statistics, such as mean, median, standard deviation, frequency, will be calculated to demonstrate patients' baseline demographics and clinical characteristics

9.4.6 Planned Interim Analyses

We plan to perform one Interim analysis which is scheduled when the primary outcome (TUG) data is collected from 8 patients from each group (mid-way). O'Brien-Fleming approach was used to prespecify efficacy boundary. The bounds are set as p=0.0054 (df=14, t-value=3.287, or effect size=1.64) and final analysis with bound p=0.0492 (df=26, t-value=2.06, effect size=0.78).

9.4.7 Sub-Group Analyses

N/A

9.4.8 Tabulation of individual Participant Data

N/A

10. REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

All participants will receive a verbal explanation of the purposes, procedures and potential risks of the study and of their rights as research participants in terms appropriate for their level of comprehension. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

If the investigator feels the individual's capacity to give informed consent is questionable, they will not participate in the study.

The consent form contains all required elements.

10.1.2 Consent for minors when they reach the age of majority

N/A

10.1.3 Telephone consent

N/A

10.1.4 Telephone assent

N/A

10.1.5 Participation of Subjects who are/become Decisionally Impaired

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **2.3.3**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team(ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to <study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities>. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. The stopping of the study will occur in the event of a major medical event, such as cardiorespiratory or cerebrovascular event or other major organ acute dysfunction requiring elevation in level of care. Ketogenic diet has been widely studied in various populations including Parkinson's Disease and these studies have not demonstrated an elevated the risk occurance of this type of event.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.3 CONFIDENTIALITY AND PRIVACY

All research activities will be conducted in as private a setting as possible.

Data will be stored using codes that we assign. Data will be kept in password-protected computers and locked storage. Only study investigators and auditors will have access to the data. Medical records will be stored in locked storage or the electronic medical record and Medical Records Department per NIH procedures.

The PI will train study staff to respect the privacy and confidentiality of NIH employees/staff through staff discussions and written branch/section procedures. The subjects will be informed that these results may be published for scientific purposes, provided their identity is not revealed. NIH may have access to the study data for auditing purposes.

Upon study completion, de-identified results may be posted to: https://clinicaltrials.gov.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data will be maintained on secure, password-protected NIH computers and servers for future use.

10.5 SAFETY OVERSIGHT

Safety oversight will be provided by the Principal Investigator.

10.6 CLINICAL MONITORING

N/A

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance monitor

The NINDS Quality Assurance (QA) Audit Committee will periodically monitor the protocol.

Quality assurance plan

This protocol will undergo periodic review by the QA Audit Committee as outlined in the NINDS QA Standard Operating Procedure (SOP).

This protocol will undergo random review by the NINDS Quality Assurance (QA) Office as outlined in the NINDS QA Standard Operating Procedure. The purpose of the QA audit is to assess compliance with applicable regulatory requirements, good clinical practice guidelines, NINDS/NIH policies, as well as to provide recommendations for improving the management of clinical research data. The protocol will be audited according to the decision algorithm as described in the NINDS SOP.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.
All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.
Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.
Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into CiStar a 21 CFR Part 11-compliant data capture system provided by the NINDS. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents will be retained as per the NIH Intramural Records Retention Schedule.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to NINDS Program Official and section chief. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:
National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information

Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. De-identified data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting NINDS study PI Dr. Debra Ehrlich and will be available in NINDS public repository: data.ninds.nih.gov.

10.10.2 Genomic Data Sharing Plan

N/A

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

N/A

10.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the <specify NIH Institute or Center (IC)> has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AD	Alzheimer's Disease
AE	Adverse Event
AI	associate investigator
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
ECR	extensor carpi radialis
eCRF	Electronic Case Report Forms
FCR	flexor carpi radialis

FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intention-To-Treat
LFD	Low Fat Diet
LSMEANS	Least-squares Means
MCT-KD	medium chain triglyceride ketogenic diet
MD	Mediterranean diet
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NMSS	Non-Motor Symptom Severity scale
NIH	National Institutes of Health
NK	nutritional ketosis
PD	Parkinson's Disease
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAD	Standard American Diet
SAE	Serious Adverse Event
SRT	simple reaction time
SOP	Standard Operating Procedure
TUG	Timed Up & Go test
UD	usual diet
UP	Unanticipated Problem
UPDRS	Unified Parkinson's Disease Rating Scale

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