Microbiome-Gut-Brain Axis and Dietary Interventions in Parkinson’s Disease

by

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_Science-

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Abstract

Parkinson’s disease (PD) is a complex neurodegenerative disorder with a multifaceted etiology involving the nervous, gastrointestinal, and immune systems. Although motor dysfunctions are the most notable clinical feature of PD, the disease is also associated with various non-motor symptoms, many of which occur before the onset of motor signs. Prodromal gastrointestinal symptoms, alterations in the gut microbiome, and trans-vagal propagation of α-synuclein aggregates from the gut to the brainstem suggest a possible intestinal origin for PD. Another pathophysiological feature of PD is the inefficient utilization of glucose by neuronal cells, which leads to bioenergetic deficits in the brain. Current PD therapies only provide symptomatic relief without addressing the underlying causes. Therefore, dietary interventions have emerged as a promising therapeutic approach that can target multiple disease mechanisms. For example, the Mediterranean diet (MeDi) and the ketogenic diet (KD) have shown promise in alleviating gastrointestinal and bioenergetic deficits in PD, respectively, while providing symptomatic relief. The KD provides an alternative source of energy to the brain, while the MeDi replenishes the population of short-chain fatty acid (SCFA)-producing bacteria. However, classical KDs may unfavorably alter the gut microbiome and decrease SCFA levels. Therefore, combining the principles of the MeDi and KD may allow us to harness the potential benefits of both dietary interventions while maintaining gut health. This thesis will analyze the literature on PD pathophysiology, particularly with respect to the microbiome-gut-brain axis, as well as the biological mechanisms of dietary interventions to propose a mechanistic framework for the application of Mediterranean-ketogenic diets in PD. Additionally, to investigate the safety and feasibility of Mediterranean-ketogenic diets (MeDi-KD) and MeDi diets supplemented with
medium-chain triglycerides (MeDi-MCT) in PD patients, an open-label, randomized, cross-over clinical trial was designed, the protocol for which will also be presented.
Lay Summary

Parkinson's disease (PD) is a neurological disorder that affects the nervous, gastrointestinal, and immune systems. The causes of PD are complex but some factors that contribute to it include issues with energy utilization by brain cells and likely alterations in the microbial residents of the gut. Current treatments only provide relief from symptoms without addressing these contributing factors. However, dietary interventions like the Mediterranean and the ketogenic diets have emerged as a promising approach to manage PD, since they have the potential to reduce the gut and brain-energy abnormalities, respectively. By integrating these diets, it may be possible to combine their benefits. To investigate this, a new clinical trial was designed to test the safety and practicality of Mediterranean-ketogenic diets in patients with PD, the details of which are presented in here. This thesis will also examine the mechanisms of these diets and how they may benefit patients with PD.
Preface

I wrote the entirety of chapters 1 (Introduction) and 2, while the protocol written in chapter 3 was done primarily by Dr. Silke Appel-Cresswell and myself. The initial idea for using medium-chain triglyceride (MCT) oil as a ketogenic intervention in patients with Parkinson’s disease was proposed by me. I assisted Dr. Appel-Cresswell, the other study co-investigators, and Dr. Sacheli in writing the grant proposal for the clinical trial proposed in chapter 3 by researching the relevant biological mechanisms and the specifics of each intervention. In addition to writing the protocol for this clinical trial I also contributed to its commencement by obtaining the Clinical Research Ethics Board’s approval, obtaining Vancouver Costal Health Research Institute’s operational approval, devising an informed consent form, communicating with various third-party companies and ordering the relevant study materials (including the MCT oil supplement and the ketone monitoring system), constructing the Research Electronic Data Capture (REDCap) website for data entry and management, and creating recruitment materials and assessment tools such as study posters, study journals, and qualitative interview questions (based on materials provided by Dr. Tamara Cohen).

Though none of the chapters presented in this thesis have been published yet, a version of chapter 3 is planned for publication.

The protocol presented in chapter 3 has been approved by University of British Columbia’s Clinical Research Ethics Board under the name: Ketogenic Diet Interventions in Parkinson’s Disease: Safeguarding the Gut Microbiome (KIM), with the reference number H21-03747.
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<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>Alzheimer’s Disease Assessment Scale – Cognitive Subscale</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse Events</td>
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<tr>
<td>aHEI</td>
<td>Alternative Healthy Eating Index</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic Lateral Sclerosis</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>AS</td>
<td>Starkstein Apathy Scale</td>
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<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>BDI-II</td>
<td>Beck Depression Inventory – II</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>BHB</td>
<td>Beta-Hydroxybutyrate</td>
</tr>
<tr>
<td>C. elegans</td>
<td>Caenorhabditis elegans</td>
</tr>
<tr>
<td>C-DHQ</td>
<td>Canadian version of Dietary History Questionnaire – II</td>
</tr>
<tr>
<td>CRA</td>
<td>Clinical Research Associate</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Forms</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>DHQ-II</td>
<td>Diet History Questionnaire II</td>
</tr>
<tr>
<td>DMCBH</td>
<td>Djavd Mowafaghian Centre for Brain Health</td>
</tr>
<tr>
<td>DMNX</td>
<td>Dorsal Motor Nucleus of the Vagus Nerve</td>
</tr>
<tr>
<td>DSS</td>
<td>Dextran Sulfate Sodium</td>
</tr>
<tr>
<td>E.Coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ETC</td>
<td>Electron Transport Chain</td>
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<tr>
<td>FFAR</td>
<td>Free Fatty Acid Receptor</td>
</tr>
<tr>
<td>FFS</td>
<td>Fatigue Severity Scale</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography Mass Spectroscopy</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
</tr>
<tr>
<td>HC</td>
<td>High Carbohydrate</td>
</tr>
<tr>
<td>HCAR</td>
<td>Hydroxycarboxylic Acid Receptor</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone Deacetylase</td>
</tr>
<tr>
<td>HDL</td>
<td>High-Density Lipoproteins</td>
</tr>
<tr>
<td>HF</td>
<td>High Fat</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity C-Reactive protein test</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowl Disease</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable Bowl Syndrome</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<tr>
<td>iNOS</td>
<td>Inducible Nitric Oxidase Synthase</td>
</tr>
<tr>
<td>K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>Potassium Sensitive ATP channels</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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</tr>
<tr>
<td>KB</td>
<td>Ketone Bodies</td>
</tr>
<tr>
<td>KD</td>
<td>Ketogenic Diets</td>
</tr>
<tr>
<td>LAS</td>
<td>Lysosomal Autophagy System</td>
</tr>
<tr>
<td>LB</td>
<td>Lewy Bodies</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long-Chain Fatty Acid</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
</tr>
<tr>
<td>LFHC</td>
<td>Low-Fat High Carbohydrate</td>
</tr>
<tr>
<td>LPB</td>
<td>LPS-Binding Protein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Leucine Rich Repeat Kinase 2</td>
</tr>
<tr>
<td>MAD</td>
<td>Modified Atkins Diet</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild Cognitive Impairments</td>
</tr>
<tr>
<td>MCT</td>
<td>Medium-Chain Triglycerides</td>
</tr>
<tr>
<td>MCFA</td>
<td>Medium-Chain Fatty Acids</td>
</tr>
<tr>
<td>MDS</td>
<td>Movement Disorder Society</td>
</tr>
<tr>
<td>MDS-UPDRS</td>
<td>Movement Disorder Society – Unified Parkinson’s Disease Rating Scale</td>
</tr>
<tr>
<td>MeDi</td>
<td>Mediterranean Diet</td>
</tr>
<tr>
<td>MeDi-KD</td>
<td>Mediterranean-Ketogenic Diet</td>
</tr>
<tr>
<td>MeDi-MCT</td>
<td>Mediterranean diet with MCT oil supplementation</td>
</tr>
<tr>
<td>MIND</td>
<td>Mediterranean-DASH Diet Intervention for Neurodegenerative delay</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced NAD</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear Factor Kappa B</td>
</tr>
<tr>
<td>NIHTB-CB</td>
<td>National Institute of Health Toolbox – Cognitive Battery</td>
</tr>
<tr>
<td>NLRP3</td>
<td>NOD-Like Receptor Family Pyrin Domain-Containing 3</td>
</tr>
<tr>
<td>NNHPD</td>
<td>Natural and Non-prescription Health Products Directorate</td>
</tr>
<tr>
<td>NOD</td>
<td>Nucleotide Oligomerization Domain</td>
</tr>
<tr>
<td>OTC</td>
<td>Over the Counter</td>
</tr>
<tr>
<td>PAS</td>
<td>Parkinson Anxiety Scale</td>
</tr>
<tr>
<td>PASIPD</td>
<td>The Physical Activity scale for Individuals with Physical Disabilities</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s Disease</td>
</tr>
<tr>
<td>PDQ-39</td>
<td>Parkinson’s Disease Questionnaire – 39</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PGIC</td>
<td>Patient Global Impression of Change</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PINK-1</td>
<td>PTEN induced kinase 1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PPRC</td>
<td>Pacific Parkinson Research Centre</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and Tensin</td>
</tr>
<tr>
<td>RBD</td>
<td>Rapid Eye-Movement Behavior Disorder</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RD</td>
<td>Registered Dietitian</td>
</tr>
<tr>
<td>REDCap</td>
<td>Research Electronic Data Capture</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-Chain Fatty Acids</td>
</tr>
<tr>
<td>SIRT</td>
<td>Sirtuins</td>
</tr>
<tr>
<td>SNCP</td>
<td>Substantia Nigra Parc Compacta</td>
</tr>
<tr>
<td>TAC</td>
<td>Total Antioxidant Capacity</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-Like Receptors</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-alpha</td>
</tr>
<tr>
<td>UAE</td>
<td>Unlisted/unexpected adverse event</td>
</tr>
<tr>
<td>UBC CREB</td>
<td>University of British Columbia Clinical Research Ethics Board</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
</tr>
<tr>
<td>WOCBP</td>
<td>Women of Childbearing Potential</td>
</tr>
</tbody>
</table>
Acknowledgements

I am filled with unending gratitude towards my supportive supervisor, Dr. Silke Appel-Cresswell, and the students and staff at the Appel-Cresswell lab for their continuous encouragement and unwavering support throughout my academic journey. I would like to extend my heartfelt appreciation to the members of my supervisory committee who generously dedicated their time and guided me through my graduate studies.

Furthermore, I would like to express my sincere thanks to my loving partner and family, whose unconditional support made even the steepest of hills easy to climb.
To My Loving Partner and Family
Chapter 1: Introductions

1.1 Epidemiology

Parkinson’s disease (PD) is the fastest growing neurological disorder affecting around 2% of the world’s population above the age of 65, thereby making it the second most common neurodegenerative disorder after Alzheimer’s disease (AD).\textsuperscript{1–3} The prevalence of PD is estimated at around 572 cases per 100,000 in those above the age of 45 across North America.\textsuperscript{4} The number of PD cases in the US was estimated to be around 680,000 in 2010 and projected to reach 1.2 million cases by 2030.\textsuperscript{4} In Canada, the number of people living with diagnosed parkinsonism in 2014 was estimated at 88,000 with an incident rate of 52.6 per 100,000.\textsuperscript{5} The incidence and prevalence of the disease increases sharply in those above the age of 60, and mortality rates can increase to double the rate of the general population, a decade after the onset of the disease.\textsuperscript{1}

The sharp increase in the rate of PD across the world has been attributed to many factors including an increase in the aging population, longevity, and industrialization.\textsuperscript{2} Given that aging is perhaps the most prominent risk factor for PD, the increase in life expectancy throughout the past decades has contributed to the upward trend of PD prevalence.\textsuperscript{2} Additionally, the rate of industrialization is positively correlated with the rates of PD, potentially due to an increase in exposure to toxic risk factors such as specific pesticides, solvents, and heavy metals.\textsuperscript{2}

PD is generally more common in men than in women, however, the mortality rate and the rate of disease progression is typically higher in women with PD.\textsuperscript{1,3,6} Moreover, the symptomatology of PD and response to medication also appear to be different in men and women with PD.\textsuperscript{6} These
sex-specific differences are thought to be driven by factors such as differential environmental risk exposure, potential protective effect of female hormones, sex-specific genetic mechanism and disparities in health care.\textsuperscript{1,6}

A small percentage of cases (about 5-10\%) are linked to rare monogenetic mutations; however, PD is largely considered to be idiopathic in nature.\textsuperscript{1}

1.2 Clinical manifestations

Though primarily recognized as a movement disorder, PD is also associated with a host of non-motor symptoms, some of which predate the onset of motor symptoms by many years.\textsuperscript{1,7,8} Based on the Movement Disorder Society’s (MDS) Clinical Diagnostic Criteria, the presence of motor features remains central to establishing a formal diagnosis of PD. Nonetheless, the absence of non-motor symptoms is considered a red flag making a diagnosis of PD less likely.\textsuperscript{7}

1.2.1 Motor symptoms

The main clinical features of PD include the presence of bradykinesia as the core motor symptom alongside either resting tremors or rigidity, which is the required criteria for establishing a formal diagnosis.\textsuperscript{1,7,9} Bradykinesia refers to slowness of movement that interferes with activities of daily living, especially those that require fine motor control.\textsuperscript{9} Additionally, bradykinesia can also manifest as difficulties in speech (dysarthria), loss of facial expression (hypomimia), and decreased blinking.\textsuperscript{9} Rigidity is described as increased resistance along the range of motion of a limb and is often associated with pain.\textsuperscript{9} As the disease progresses, rigidity can lead to postural deformities resulting in flexed neck and trunk postures.\textsuperscript{9} Resting tremors predominantly affect one side of the body and are particularly prevalent in the distal extremities of the patients. The resting tremors in PD can also involve the lips, chin, jaw, and legs of the
patient, which is a distinguishing feature from those with essential tremors. These tremors are typically between 4-6 Hz in frequency and dissipate with action or during sleep.

In addition to the main motor features, PD is also associated with other motor features such as postural instability and motor blocks (freezing). Postural instability, defined as the loss of postural reflexes, often develops at the later stages of the disease and is the most prominent cause of falls related to the disease. Freezing, on the other, is less common amongst patients with PD, however, it is often considered as one of the most debilitating features of the disease. It involves a sudden and transient ability to move, predominantly involving the legs, though the arms and the eyelids may also be affected.

1.2.2 Non-motor symptoms

Though primarily recognized as a motor disorder, PD is also associated with a host of non-motor symptoms that contribute significantly to the burden of the disease. The main non-motor symptoms of PD include, but are not limited to, gastrointestinal symptoms, cognitive impairments, depression and other mood-related disorders, and sleep-wake cycle dysregulations.

Cognitive impairments in patients with PD often manifest as deficits in executive function, behavioral inhibition, and memory retrieval. Cognitive symptoms are relatively mild at early-mid stages of the disease; however, as PD progresses they can become sufficiently severe to meet the requirements for diagnosis of dementia. In fact, as determined by one long-term study, dementia was present in 84% of PD patients who survived for more than 20 years. The same study also reported on the pervasiveness of hallucinations and psychotic symptoms, which were recorded in 74% of the patients.
The most established PD-associated mood disorders include depression, apathy, and anxiety. Importantly, mood-related symptoms, particularly depression, are often described by PD patients as having the greatest impact on their quality of life, yet they seldom receive sufficient attention. One study examining depression in 114 PD patients reported that 40% of those screened positive for depression (approximately 27% of the overall participants) were never treated with antidepressant or referred for further psychiatric evaluation.

The most common sleep disorder in PD is rapid eye-movement sleep behavior disorder (RBD), which is seen in about 33% of patients. RBD is characterized by an increase in violent dream content that is associated with the individual ‘acting out’ their dreams. The case of RBD is of particular interest as it typically occurs years before the onset of motor symptoms and is one of the strongest predictors of later development of PD.

Lastly, constipation is the most common gastrointestinal symptom associated with PD, which similar to RBD, predates the onset of motor symptoms by years. Constipation in PD can be caused by either slow colonic transit time or anorectal dysfunction. Interestingly, constipation due to the former cause has been linked with faster disease progression as compared to constipation due to anorectal dysfunction. Moreover, severe constipation early in the disease predicts a faster progression rate to dementia as compared to those with minor or no constipation. The other gastrointestinal features of PD include excessive oral saliva levels (despite lower saliva production), dysphagia, and gastroparesis characterized by vomiting, lack of appetite, bloating, and weight loss.

1.3 Neurobiology and pathophysiology
The two hallmarks of PD neuropathology are selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC) and widespread presence of intracellular protein
aggregates known as Lewy bodies.\textsuperscript{1,21} Normally, the dopaminergic neurons of the SNPC project to the striatum and modulate the activity of the motor circuit.\textsuperscript{22} Loss of dopaminergic inputs to the motor circuit is associated with deficits in control of action and goal-directed behavior.\textsuperscript{1,22}

The presence of Lewy bodies (LB), however, is more widespread and is not exclusive to dopaminergic neurons.\textsuperscript{1,21} They are initially formed in monoaminergic and cholinergic neurons of the brainstem and in neurons of the olfactory bulb; however, as the disease progresses they will spread to other brain regions such as the limbic structures and the neocortex.\textsuperscript{21}

The main component of LBs is the $\alpha$-synuclein protein.\textsuperscript{1,23} The physiological role of $\alpha$-synuclein is not completely understood; however, it is thought to be involved in various aspects of cell function including synaptic vesicle dynamics, mitochondrial function, and intracellular trafficking.\textsuperscript{1,23,24} Disruptions in the normal proteostasis of $\alpha$-synuclein – that is, increased protein production due to gene overexpression or impairments in cellular systems involved in protein degradation – lead to accumulation of the protein and ultimately, formation of insoluble $\alpha$-synuclein fibrils.\textsuperscript{23}

Of particular interest are the disruptions in the lysosomal autophagy system (LAS) which is involved in the clearance of oligomeric assemblies of $\alpha$-synuclein and maintaining the normal proteostasis status.\textsuperscript{25} Several studies have found that genetic variations that inhibit LAS function are associated with increased $\alpha$-synuclein aggregation.\textsuperscript{26,27} Moreover, presence of $\alpha$-synuclein inclusions are associated with abnormalities in biomarkers of the LAS dysfunction in the substantia nigra of patients with PD and experimental models of PD.\textsuperscript{28} These strands of evidence point towards a pathological cycle wherein disruptions of protein degradation systems lead to aggregation of $\alpha$-synuclein and $\alpha$-synuclein aggregates cause further disruptions in protein degradation systems.\textsuperscript{25}
Furthermore, it has been shown that LAS impairments can lead to exosome-mediated secretion of α-synuclein into the extracellular space, which is subsequently endocytosed by other neurons. This provides support for the prion-like spread hypothesis which posits that α-synuclein aggregates can spread from the site of formation to neurons of other regions and seed aggregation of endogenous α-synuclein in their new environment. The prion-like spread model is supported by neuropathological examinations of Braak et al., wherein pathological α-synuclein aggregates were found to spread from their site of origin to distant brain regions via intraneuronal and trans-neuronal transport mechanisms.

In summary, disruptions of the normal proteostasis of α-synuclein proteins and their subsequent aggregation into oligomeric fibrils is associated with a number of cytotoxic events such as impairments in brain energetics, oxidative stress, and neuroinflammation. Moreover, each of these pathophysiological effects amplifies the cytotoxic state of the cell by promoting destructive feedforward cycles. The following sections will examine each of these cytotoxic events and their implication in PD pathology in more detail.

1.3.1 Bioenergetics deficits

A prominent feature of PD pathology is impairments in brain energetics and hypometabolism. Evidence from Positron Emission Tomography (PET) imaging studies have shown that patients with PD exhibit a deficit in glucose metabolism in several brain regions including the striatum and the frontal cortices and that this deficiency is correlated to the severity of motor and cognitive symptoms. This impairment has been attributed to a dysfunctional mitochondrial complex I, a component of the electron transport chain. The disruption of complex I function leads to a less efficient pumping of protons across the inner mitochondrial membrane into the intermembrane space, which reduces the proton gradient that is needed to produce ATP.
is an abundance of evidence that supports the involvement of mitochondrial dysfunction in PD pathology. Firstly, neurotoxins that inhibit mitochondrial function, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, are widely used to induce parkinsonian features in animal models of PD.\textsuperscript{34} Second, many genetic mutations that are linked with familial forms of PD, such as SNCA – which encodes α-synuclein - and Leucine Rich Repeat Kinase 2 (LRRK2), are associated with mitochondrial malfunctions such as fragmentations and reduction of mitochondrial biogenesis.\textsuperscript{34,37} Moreover, genetic mutations associated with early onset of Parkinson’s disease, such as phosphatase and tensin (PTEN) induced kinase 1 (PINK-1), are thought to contribute to mitochondrial dysfunction by disrupting mitophagy, the process of degrading damaged mitochondria.\textsuperscript{34,38} Third, environmental risk factors associated with development of PD – such as exposure to pesticides (e.g., rotenone and paraquat) – exert their toxic effects by interfering with mitochondrial function or damaging the mitochondrial DNA.\textsuperscript{34} This raises the question of how mitochondrial dysfunction contributes to PD pathology and whether this damage can be reversed or prevented.

The main downstream effects of mitochondrial dysfunction include insufficient production of ATP as the main energy source of the body and exerting oxidative stress through the generation of radical species.\textsuperscript{34} The dopaminergic neurons of the nigrostriatal pathway are particularly vulnerable to energy depletion and oxidative damage mainly due to their large energy expenditure and production of dopamine metabolites that exert oxidative stress on the cell even in normal conditions.\textsuperscript{39} Consequently, mitochondrial damage that leads to a depletion of energy resources and exertion of oxidative stress, greatly contributes to the degeneration of dopaminergic neurons and the consequences that ensue.\textsuperscript{39}

\textbf{1.3.2 Neuroinflammation and oxidative stress}
Another pathological feature of PD is the persistent presence of an immune response, both centrally and peripherally, which leads to the inflammation of the brain and neuronal cell death.\(^4\) The central immune response involves the activation of the brain’s resident innate immune cells, microglia and the subsequent initiation of a neuroinflammatory response.\(^1,4,41\) Under physiological conditions, microglia exhibit an inactive phenotype wherein they survey the environment for ‘danger’ signals and release anti-inflammatory and neurotrophic factors.\(^41\) Once the microglia are presented with pathogens or epitopes that signal tissue damage, they become activated and start releasing proinflammatory factors that further engage the immune system.\(^40,41\) PET neuroimaging studies have revealed that patients with PD exhibit greater microglial activation than healthy controls, even in those who are at the earlier stages of the disease.\(^42\)

Several pathological mechanisms are thought to be involved in the activation of microglia and the subsequent neuroinflammatory response in PD.\(^40\) First, direct activation of microglia by extracellular α-synuclein aggregates has been shown in several studies, and linked with cytotoxic effects such as production of proinflammatory cytokines.\(^40,43\) Second, the death of dopaminergic neurons and the release of their intracellular content into the extracellular space can also signal the activation of microglia.\(^43\) Degeneration of dopaminergic neurons is associated with release of neuromelanin, a by-product of catecholamine catabolism, into the extracellular space which is then engulfed by the microglia, leading to their activation and an increase in expression of proinflammatory genes.\(^44\) These genes typically code for pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), which act as signal amplifiers to further engage the immune system, and proteins with antimicrobial activity such as inducible nitric oxidase synthase (iNOS), which releases NO.\(^40,41,43\) Moreover, activation of microglia is also associated with upregulation of the NADPH-oxidase system and release of
reactive oxygen species (ROS).\textsuperscript{41,45} It is interesting to note that a persistent neuroinflammatory response will cause microglia to reprogram their metabolic pathway such that they become predominantly dependent on the less efficient glycolysis instead of their normal oxidative phosphorylation metabolism, thereby further limiting the energy availability to the neurons.\textsuperscript{32} Furthermore, the activated microglia can also act as antigen-presenting cells and present α-synuclein to the infiltrating adaptive immune cells such as T cells.\textsuperscript{46} The activated T cells, in turn, can release proinflammatory cytokines, thereby aggravating the existing neuroinflammation and cell death.\textsuperscript{40,46} Infiltration of adaptive immune cells into the brain has been linked with disruptions of blood brain barrier (BBB) due to aging and existence of a peripheral inflammatory response.\textsuperscript{40,47} It has been suggested that chemokines produced by the humoral immune cells can direct leukocytes towards an immune signal and increase the permeability of the BBB.\textsuperscript{48} Interestingly, chemokines production induced by lipopolysaccharides (LPS) has been implicated in patients with PD.\textsuperscript{48} Infiltration of LPS into the systemic circulation has been linked with gut dysbiosis and inflammation, which is discussed in more detail in subsequent sections. Interestingly, the manifestation and severity of many non-motor symptoms of PD seem to be associated with levels of .\textsuperscript{49,50} For instance, in one study examining the relationship between proinflammatory cytokines and non-motor symptoms of PD, it was shown that higher levels of TNF-α was significantly correlated with poorer performance in cognitive tasks, especially those that involve executive function and language.\textsuperscript{49} Moreover, TNF-α accounted for 25% of variability in composite cognitive measures in multivariate regression analyses.\textsuperscript{49} Similarly, higher levels of TNF-α and IL-1β were also associated with more severe depression symptoms and dysregulations in the sleep cycle.\textsuperscript{49,51}
As previously mentioned, dopaminergic neurons of SNPC seem to be particularly vulnerable to oxidative stress as even in physiological conditions they produce large quantities of ROS due to processes involved in synthesis of dopamine. The oxidative stress on dopaminergic neurons is exacerbated with PD pathology, as more ROS are released due to mitochondrial dysfunctions and neuroinflammation. This is especially evident in mutations of the DJ-1 gene, which encodes an important antioxidant and is associated with a rare form of autosomal recessive juvenile PD. Another downstream effect of oxidative stress on dopaminergic neurons is the depletion of lysosomes and impairment of LAS, which disrupts the effective clearance of aggregated proteins and consequently, leads to further generation of cytotoxic α-synuclein fibrils.

1.4 Gastrointestinal Involvement and Gut Dysbiosis

The presence of α-synuclein fibrils within LB inclusions found in the enteric nervous system and their potential to be retroactively transported to the brain via the vagus nerve, the early onset of gut-related symptoms in PD patients, and the well-established gut microbiome disturbances, all suggest that the gastrointestinal system is heavily involved in the pathophysiology and even the pathogenesis of PD.

1.4.1 Intestinal Origin of PD

The hypothesis postulating the GI tract as the origin of idiopathic PD was first proposed by Braak et al., and was further developed by later studies. Braak et al., hypothesized that the pathogenic agent, in this case α-synuclein aggregates, can propagate via axonal and trans-neuronal processes from the axon terminals of parasympathetic neurons to infiltrate vulnerable brainstem nuclei such as the dorsal motor nucleus of the vagus nerve, cranial nerve X (DMNX). A later study confirmed this hypothesis by demonstrating that α-synuclein particles
introduced to the intestinal wall of rats in form of PD brain tissue lysates can be transferred to the
DMNX via the vagus nerve.\textsuperscript{61} Moreover, it has also been shown that severing the vagus nerve
prevents the spread of the $\alpha$-synuclein aggregates from the gut to the brain and diminishes the
associated behavioral deficits in rats.\textsuperscript{62} $\alpha$-synuclein fibrils have been found in the enteric nervous
system of those with PD, in some cases pre-dating the official diagnosis by 20 years.\textsuperscript{54} Moreover,
the enteric $\alpha$-synuclein stains are correlated with measures of intestinal barrier integrity,
suggesting the exposure of the intestinal neuronal tissue to environmental toxins and luminal
proinflammatory substances as a potential mechanism for $\alpha$-synuclein aggregation.\textsuperscript{63}

1.4.2 Gut Dysbiosis

Gut dysbiosis is a well document feature of PD pathology with altered taxonomic composition,
decreased Short-Chain Fatty Acids (SCFA) production, impaired gut-barrier integrity, and
increased pro-inflammatory capacity as the key characteristics.\textsuperscript{24,56–60} The potential role of gut
dysbiosis in PD pathogenesis has been demonstrated in several pre-clinical studies using germ-
free or conventionally colonized PD animal models. For instance, it has been shown that
transplantation of fecal matter from human donors with PD to alpha-synuclein overexpressing
germ-free mice leads to a greater exacerbation of PD features and inflammation as compared to
fecal matter transplanted from healthy controls.\textsuperscript{64} Moreover, $\alpha$-synuclein overexpression led to
deterioration of motor function, constipation, neuroinflammation, and $\alpha$-synuclein aggregation
only in the mice with an intact gut microbiome and not in their germ-free counterparts.\textsuperscript{64}
Similarly, another study has demonstrated that fecal matter transplantation from normal mice to
mice treated with MPTP leads to improvements in motor function, striatal dopamine and
serotonin levels, and inflammatory responses\textsuperscript{65}. The next sections will examine the clinical
evidence pertaining to different aspects of gut dysbiosis in PD in more details.
1.4.2.1 Microbiome composition

Taxonomic profiling of the gut microbiome using 16S rRNA sequencing has shown significant compositional differences between patients with PD and control individuals\(^{24,57,58,60,66-69}\). However, due to the heterogeneity of sample collection and analysis protocols across various studies, the effect size of these differences remain relatively modest with the majority of variance being explainable by study methodology and geographical location\(^70\). Nonetheless, some of the differences in the relative abundance of various bacterial taxa between patients with PD and control individuals have been consistently highlighted across multiple studies including an increase in the relative abundance of the genera *Akkermansia* (and the Verrucomicrobiaceae family, to which it belongs)\(^{24,56,59,60,66-69}\), *bifidobacterium*\(^{24,56,59,60,69}\) and *lactobacillus*\(^{60,67,69,71}\), and a decrease in the abundance of Lachnospiracea family (particularly the *Roseburia* genus)\(^{24,57,59,60,66,68,71}\) and the *Prevotella*\(^{56,60,67,71}\), *Faecalibacterium*\(^{24,57,60,66,68,69,71}\) and *Blautia*\(^{24,68}\) genera.

Despite the relative consistency of these findings across several studies, the functional implications of these alterations are yet to be fully understood. For instance, *Akkermansia* is typically considered to be a probiotic associated with positive health outcomes such as increased short-chain fatty acids (SCFA) production and improved insulin sensitivity\(^{70,72,73}\). However, in the absence of sufficient dietary fiber, *Akkermansia* is thought to be associated with an unhealthy increase in the decomposition of the intestinal mucin layer and consequently, impaired gut-barrier integrity\(^{57,74}\). Moreover, the relative abundance of *Akkermansia* has been found to be elevated in patients with RBD\(^70\), a prodromal condition for PD, and increasing with PD disease progression\(^57\), suggesting a potential contributory role in conversion of RBD to PD.
Additionally, the functional implications of bacterial taxa likely depend on the specific species and strains and the interactions between them. For instance, although the genus *Prevotella* is commonly reported to be decreased in patients with PD, recent studies utilizing shotgun metagenomic sequencing to investigate gut dysbiosis at the species level have found that the pathogenic species of this genus are collectively found at higher relative abundances, while the putatively beneficial species *Pretovella copri* was decreased.\textsuperscript{75} Moreover, the differential relative abundance of *Akkermansia* was found to be dependent on the geographical location from which the samples were obtained.\textsuperscript{24,69,75} Thus, the functional implication of taxonomic alterations in the gut microbiota will depend on a multitude of factors, including the underlying pathological condition, specific species and strains and their intercommunications, and variability in lifestyles such as diet and geography.\textsuperscript{70}

The following sections will review the functional implications of PD gut dysbiosis, specifically the decrease in SCFAs, the impairment of the intestinal barrier integrity, and the ensuing enteric and systemic inflammation in more details.

### 1.4.2.2 SCFAs depletion

SCFAs are the by-products of bacterial fermentation of complex carbohydrates and to a lesser extent, branch-chained amino acids.\textsuperscript{76} The three main SCFAs produced by the gut microbiota include acetate, butyrate, and propionate existing in a 60:20:20 mM ratio.\textsuperscript{77}

SCFAs are one of the main communication pathways between the gut microbiome and the brain, as they can initiate signaling pathways by binding to G-Protein coupled receptors (GPCR), modify histone deacetylase complexes, and modulate immune response.\textsuperscript{78} The primary SCFA GPCRs include the free fatty acid receptors (FFAR) 2 and 3, and the GPCR hydroxycarboxylic
acid receptor 2 (HCAR 2) (also known as GPR109A). The downstream effects of the activation of these receptors include the induction of intestinal gluconeogenesis, activation of the sympathetic nervous system, regulation of cellular metabolism, and modulation of immune responses through altering the function and maturation of microglia. Moreover, SCFA are the main energy source for intestinal colonocytes, and their depletion is associated with increased gut leakiness and impaired gut-barrier integrity.

SCFA deficiency has been widely reported in patients with PD and is believed to be a major consequence of gut dysbiosis in PD. In a study comparing the SCFA profile and bacterial composition of the microbiome in PD patients and their age-matched controls, it was found that the SCFAs, especially butyrate, are significantly reduced in PD patients. Additionally, some components of SCFA metabolic pathway, including butyrate kinase which catalyzes a reversible reaction between butyrate and butanyol-phosphate are also found to be reduced in PD patients. Furthermore, lower fecal SCFA levels (particularly butyrate) has been associated with increased severity of several PD symptoms including postural instability, cognitive impairments, constipation, and depression.

1.4.2.3 Intestinal permeability

The intestinal barrier separates the luminal content of the gut from the intestinal and systemic circulation. Loss of the intestinal barrier’s integrity can lead to a condition known as “leaky gut”, wherein the intestinal and systemic circulations become exposed to the proinflammatory luminal content of the gut such as lipopolysaccharides (LPS). Importantly, SCFAs influence the integrity of the intestinal barrier through GPCR signaling cascades and HDAC-inhibition, in addition to being an energy source for the intestinal colonocytes. Therefore, depletion of SCFA-producing bacteria and the subsequent reduction of luminal SCFA content can lead to a
loss of intestinal barrier integrity, and consequently, a chronic low-grade inflammatory response will ensue.77 Another potential mechanism for increased gut permeability in PD is the dysbiosis of the gut microbiota, particularly an increase in the relative abundance of mucin degrading bacteria such as Akkermansia.

“Gut leakiness” is a well-documented condition in patients with PD and has been associated with increased levels of gut inflammation and α-synuclein aggregation.63,84,85 For instance, it has been shown that the colonic mucosal specimens of patients with PD obtained via colonic biopsies display an altered morphology in terms of the expression and distribution of tight junction proteins as compared to healthy controls.85 Moreover, patients with PD have significantly higher levels of Zonulin and alpha-1 antitrypsin (biomarkers of intestinal permeability) as compared to age-matched healthy controls.84 Zonulin is a modulator of tight junction proteins and an increase its concentration is indicative of disruptions in the gut barrier integrity.84 Similarly, higher levels of alpha-1-antitrypsin, which is a protease inhibitor, is reflective of mucosal barrier dysfunction. Higher levels of zonulin were also reported in both stool and serum samples of patients with PD as compared to healthy controls in a more recent study, further reinforcing the occurrence of “leaky gut” in PD.86

Another study established that not only do patients with PD exhibit greater gut permeability as compared to healthy controls, but also that gut hyperpermeability in PD patients is significantly correlated with greater mucosal staining for α-synuclein, nitrotyrosine (a measure of oxidative stress), and Escherichia coli (E.coli) bacteria.63 The latter is significant as it confirms that LPS-producing bacteria can invade the intestinal epithelial cells, where they can induce inflammatory responses.

1.4.2.4 Gut inflammation
Increased gut permeability in patients with PD leads to the infiltration of intestinal epithelial tissue by bacteria and toxins. Of the infiltrating bacteria, the LPS-expressing gram-negative bacteria (e.g., *E. coli*) are of particular interest, as LPS can bind to Toll-Like receptors (TLR; specifically TLR4) to mount an immune response.40,70,77

TLR4 receptors are expressed abundantly in various cell types, including the enteric immune cells, and their activation may lead to an immune response that further aggravates gut dysbiosis, colonic hyperpermeability, and α-synuclein aggregation.87 Furthermore, the increased permeability of the gut barrier exposes the systemic circulation to endotoxins, which can induce a more widespread inflammatory response, ultimately leading to neuroinflammation and neurodegeneration.87

One potential mechanism through which the activation of TLR4 receptors can trigger neuroinflammation is through signaling pathways that lead to the expression of un-cleaved proinflammatory proto-cytokines such as pro-IL-1β and pro-IL8, via the nuclear factor kappa B (NF-κB) pathway.88 Subsequently, these proto-cytokines, in presence of a secondary signal such as oxidative stress89 or misfolded α-synuclein aggregates90, can induce the formation of nucleotide oligomerization domain (NOD)-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome and activation of caspase 1, which is required for cleaving the proto-cytokines into their active form.88 The active pro-inflammatory cytokines (e.g., IL-1β etc.) will in turn cause sustained neuroinflammation, which contributes to α-synuclein aggregation, ROS production, and further aggravation of the inflammatory response, thereby creating a perpetual pathological cycle that ultimately leads to neurodegeneration.40

Studies investigating these mechanisms in PD have determined that 1) mucosal staining for gram-negative LPS-expressing bacteria is significantly associated with serum levels of LPS
binding protein (LPB), an indicator of systemic endotoxin exposure and 2) colonic biopsies from patients with PD exhibit greater TLR4 expression with increased pathway-specific inflammatory cytokine levels such as IL-1β, as compared to controls. Interestingly, the latter study also demonstrated that injection of rotenone to TLR4-knockout mice results in milder intestinal inflammation and motor dysfunction, and lower rates of neurodegeneration. Furthermore, administration of LPS to wildtype mice has been shown to cause a rapid and persistent increase in TNF-α levels and degeneration of nigral dopaminergic neurons. Additionally, another study investigating the pro-inflammatory profile of colonic biopsies obtained from patients with PD determined that the mRNA expression level of cytokines such as TNF-α and IL-1β are significantly elevated in patients with PD as compared to controls. Intriguingly, these elevations were not correlated with measures of disease severity, gastrointestinal symptoms, or cumulative lifetime Levodopa dose. Nonetheless, the lack of such associations in this study can be attributed to the specific methodology used for determining the cytokine profile (i.e., mRNA expression rather than protein up-regulation) or assessment of PD symptom severity (using the total Unified Parkinson’s Disease Rating Scale scores).

Gut inflammation in PD has been studied using other biomarkers such as calprotectin as well. Calprotectin is a member of the S100 family of proteins and is released from neutrophils upon activation. An increase in fecal calprotectin levels implies the migration of neutrophils into the intestinal mucin layer or the lumen, thereby indicating an impairment of intestinal barrier integrity. PD patients exhibit significantly elevated calprotectin levels in both their stool and serum samples.

The other piece of evidence implicating gut inflammation in PD pathogenesis emerges from association studies linking other diseases that involve gut inflammation such as irritable bowl
disease (IBD) with PD.\textsuperscript{70} For instance, it has been reported that patients with IBD have a 28% chance of being diagnosed with PD, which is significantly higher than matched healthy controls.\textsuperscript{95} Furthermore, this study demonstrated that effective treatment of IBD with anti-inflammatory medications (anti-TNF therapy) reduced the risk for developing PD by 78\%.\textsuperscript{95} Intriguingly, both PD and IBD have been associated with a common genetic risk/protective factors. For instance the N551K and R1398H variations in the LRRK2 gene are associated with a reduced risk for developing both PD and IBD (specifically, Crohn’s disease), while the N2081D variation increases the risk of developing both diseases.\textsuperscript{96} Collectively, the evidence from these studies confirms that gut dysbiosis is associated with PD pathology, however, the exact mechanisms through which it can aggravate neurodegeneration or contribute to symptom severity are yet to be fully elucidated. It is likely that the complex interplay between taxonomic alterations, gut barrier hyperpermeability, and gut inflammation is likely to aggravate systemic pathological mechanisms, ultimately leading to neuronal degeneration.\textsuperscript{40,70} Nonetheless, regardless of the specific mechanisms involved, future therapeutic avenues being considered should bear the role of gut dysbiosis in PD pathology in mind in order to avoid causing further disruptions to this perturbed system while intervening at the level of gut dysbiosis and inflammation might ultimately provide a novel and early opportunity of intervention.

1.5 Central Questions and Objectives

As described above, PD pathophysiology involves multiple interconnected mechanisms that contribute to neurodegeneration and overall disease progression.\textsuperscript{1,10} Furthermore, the available pharmacological treatments provide transient, motor-specific symptomatic relief without addressing the underlying causes or the non-motor symptoms.\textsuperscript{1} To this end, lifestyle
interventions such as diet\textsuperscript{77,97} and exercise\textsuperscript{108,109} have gained prominence as potential disease-modifying therapeutic avenues. Dietary interventions have the capacity to address multiple disease mechanisms, thereby potentially slowing its rate of progression while providing symptomatic relief.\textsuperscript{77,97}

Recently, the Mediterranean diet (MeDi)\textsuperscript{102,103} and ketogenic diet (KD)\textsuperscript{98,100,101} have emerged as promising candidates for treating PD as – among other mechanisms- they have been shown to target gut dysbiosis\textsuperscript{110–112} and bioenergetic deficits\textsuperscript{98,107}, respectively.

The primary objective of this thesis was to start a clinical trial investigating the safety and feasibility of combined Mediterranean-ketogenic interventions with respect to gut health in patients with PD. The specific aims of this objective included 1) analyzing the literature on dietary interventions in patients with PD to determine the biological mechanisms involved, 2) integrate the evidence from studies investigating the effects of ketogenic diets on the gut microbiome in non-PD cohorts to theorize how such a diet will impact the gut health of patients with PD, and 3) to devise a protocol for a clinical trial investigating the safety of ketogenic interventions for patients with PD with respect to gut health.

The next chapter will integrate the evidence on dietary interventions in PD with a focus on biological mechanisms through which diet can influence the gut microbiome and brain health. Subsequently, Chapter 3 will outline a protocol for an open-label randomized cross-over clinical trial, which was devised to investigate the safety and feasibility of two dietary interventions in patients with PD.
Chapter 2: Mediterranean and Ketogenic Dietary Interventions in PD: Implications of the Gut Microbiome

2.1 Mediterranean Diet Interventions

MeDi typically describe a dietary pattern that has been prevalent in Greece and southern Italy since the 1960s. Generally, adherence to a MeDi involves daily intake of fruits and vegetables, particularly leafy greens, nuts and grains, and extra virgin olive oil as the primary source of fat. Moderate intake of fish and poultry is the primary source of protein in MeDi, while the intake of red meat is typically limited. Moreover, dairy products and eggs can be consumed in moderation, however, the intake of processed food and sweets should be greatly limited.

This dietary pattern results in a remarkably greater intake of dietary fiber (approximately 33 grams/day) as compared to traditional Western diet (10-15 grams/day). Dietary fiber is generally defined as indigestible and unabsorbable complex carbohydrates (i.e., with more than three monomeric units), typically found in grains, legumes, vegetables, and cereals. The high intake of dietary fiber is associated with positive alterations of the gut microbiota, which is thought to be essential in conferring the beneficial effects of MeDi. Additionally, MeDi are also rich in antioxidant containing foods such as fruits, grains, tea, and olive oil.

The following sections will review the evidence for the utility of MeDi as a therapeutic intervention in PD and the biological mechanisms through which it can influence PD pathology.

2.1.1 MeDi: Mechanisms of Action

Gut dysbiosis, characterized as compositional alterations of the gut microbiota and their associated metabolites, intestinal hyperpermeability, and gut inflammation is a key feature of PD
pathogenesis. While it is difficult to draw a causal link between the taxonomic alterations of the microbiota and the other features of PD gut dysbiosis, one can speculate about the role of SCFAs as an important mediating factor. In fact it has been shown that there is a depletion of SCFA producing bacteria in the PD gut microbiome associated with lower fecal SCFA levels including butyrate. SCFA depletion (particularly butyrate), in turn, has been linked with degradation of intestinal barrier integrity, and sustained gut inflammation.

As alluded to before, MeDi are rich in sources of dietary fiber and other indigestible carbohydrates, which are readily used by the gut microbes as an energy course. Following ingestions, dietary fiber is fermented by the gut bacteria to produce key health-promoting metabolites such as SFCA. The high intake of fiber in MeDi promotes a gut environment wherein the fiber-fermenting, SCFA-producing bacteria such as Roseburia, Faecalibacterium, and Prevotella can thrive and grow in abundance. Remarkably, these SCFA-producing bacteria (in addition to others) are commonly found to be decreased in abundance in the PD gut microbiome. Incidentally, a recent systematic review reported that the bacteria that are typically lower in abundance in PD microbiome such as Blautia glucerasea, Faecalibacterium, and Prevotella copri are found to be increased with adherence to MeDi, while those that commonly exist in a higher relative abundance such as Akkermansia, Bilophila, and Enterococcus are found at lower levels in MeDi studies. The same systematic review also reported an association between higher SCFA levels and adherence to MeDi.

SCFAs also play a crucial role in the regulation of host immune function, although the specific nature of these modulations (i.e., pro- or anti-inflammatory effects) and the mechanisms through which they operate are complex and likely depend on factors such as the
dysbiotic/eubiotic status of the gut. Nonetheless, higher MeDi scores have been associated with lower concentration of inflammatory markers including C-reactive protein and IL-6, both of which have been implicated in PD, thereby suggesting a potential anti-inflammatory role of SCFAs with MeDi in patients with PD.

Another health-promoting feature of MeDi is the high intake of foods that are rich in antioxidants bioflavonoids and polyphenols such as berries, leafy greens, and extra virgin olive oil. Polyphenols are thought to have many neuroprotective and disease-modifying effects with respect to PD, including the protection of dopaminergic neurons associated with motor function improvements through putative mechanisms such as mitigation of oxidative stress, suppression of the inflammatory responses, and inhibition of cell apoptosis.

Taken together, the evidence presented in this section suggests that MeDi can target multiple aspects of PD pathophysiology, including gut dysbiosis, oxidative stress, and colonic/systemic inflammation.

### 2.1.2 Mediterranean Diet in PD

The effects of MeDi on PD disease severity and cognitive performance have been investigated in two separate randomized controlled trials (RCT). With respect to the former, it was found that adherence to the MeDi for 10 weeks was associated with greater improvements in the total Unified Parkinson’s Disease Rating Scale (UPDRS) score as compared to the control diet (traditional Iranian diet), though no improvements were detected in the motor examinations (UPDRS part III). Furthermore, adherence to the MeDi was associated with significant improvements in the Total Antioxidant Capacity (TAC) measures obtained from serum samples, as compared to controls. Prior to this publication, the same group also reported that a 10 week
adherence to the MeDi leads to improvements in multiple domains of cognitive performance including executive function, language, attention, concentration and active memory (measured via Montreal Cognitive Assessment), in patients with PD as compared to adherence to an unspecified control diet. In spite of the promising evidence, replication of these results using more sensitive assessment tools would be highly valuable.

The associations between adherence to a MeDi and several PD outcome measures have also been investigated using a priori determination of the intake frequency of specific food groups or scoring of dietary patterns that resemble a MeDi via food intake questionnaires. For instance, using the former method Mischely et al., determined that foods that are associated with a reduced rate of PD progression include fresh fruits and vegetables, nuts and seeds, and olive oil (among others), which evidently are staples of the MeDi diet.

Similarly, dietary pattern scores reflective of a higher MeDi adherence have been found to be associated with a later PD age of onset, even when the scores were adjusted for disease duration, daily caloric intake, gender, and education. This finding was further reinforced by evidence from another study suggesting higher adherence to the MeDi is associated with lower probability of prodromal non-motor symptoms. A later study expanded on these results by determining higher adherence to the Mediterranean-DASH Diet Intervention for Neurodegenerative delay (MIND diet), a variant of MeDi specifically designed to promote brain health, is also associated with a lower risk of parkinsonism and slower disease progression rate. Furthermore, using similar methods Metcalfe-Roach et al. suggested a sex-specific mechanism for these effects, as higher adherence to the MIND diet was associated with a greater latency in the onset of PD in the female subgroup as compared to males. Additionally, higher adherence to an alternative
MeDi was found to be negatively correlated with the occurrence of prodromal PD symptoms such as constipation, excessive day-time sleepiness, and depression, further reinforcing the disease-delaying effects of MeDi on PD.\textsuperscript{123} Lastly, a recent cohort study of health professionals before and after PD diagnosis has found an inverse association between higher alternative Healthy Eating Index (aHEI) scores, and all cause mortality.\textsuperscript{124} Higher aHEI scores indicate greater intake of fruits and vegetables, whole grains, nuts and legumes, and lower intake of sugary drinks, red meat, and trans-fats, all of which are closely related to the MeDi.\textsuperscript{124}

Collectively, these results support the utility of MeDi as a viable preventative measure for PD\textsuperscript{102,105,122,123}, with a potential to reduce the rate of disease progression\textsuperscript{121,122} and improve its non-motor symptoms\textsuperscript{104,120}. Table 2.1 summarizes these studies with respect to the specific methodology used, the outcome variables, and the reported results.
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<td>Randomized Control Trial in 80 patients with PD</td>
<td>MeDi (n=40) vs. Iranian Traditional Diet (n=40) for 10 weeks</td>
<td>Significant increase in serum Total Antioxidant Capacity (TAC) measures and improved non-motor symptoms (UPDRS) with MeDi</td>
<td>The basis of TAC was not explained.</td>
<td>Paknahad et al. 104</td>
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<tr>
<td>Randomized Control Trial in 80 patients with PD</td>
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<td>Analysis of the intake frequency of various food groups. Data from 1053 patients with self-reported PD.</td>
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Table 2.1 Clinical studies of MeDi-type diets in patients with PD.

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<td>Dietary pattern analysis of 706 participants without PD at baseline over 4.6 years.</td>
<td>MIND diet, MeDi, and DASH diet</td>
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<td>Dietary pattern analysis of 167 patients with PD and 119 healthy controls</td>
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<tr>
<td>Dietary pattern analysis of 1251 individuals with PD</td>
<td>Alternative Healthy Index (similar to MeDi)</td>
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<td></td>
<td>Zhang et al.¹²⁴</td>
</tr>
</tbody>
</table>

2.2 Ketogenic Interventions

Ketogenic Diets (KD) are typically characterized as high fat, low carbohydrate diets with a moderate intake of proteins.¹²⁵,¹²⁶ The three main forms of KD are the classical KD, the modified Atkins diet (MAD), and the medium-chain triglyceride (MCT) – KD.¹²⁶ Classical KDs are by far the most restrictive of the three, with fats constituting about 90% of total energy intake, while proteins and carbohydrates providing about 8% and 2% of total energy respectively.¹²⁶,¹²⁷ MAD is considerably less restrictive, as it allows for higher intake of carbohydrates (~10% of total energy intake) and proteins (~25% of total energy intake), while limiting the contribution from
fats to about 65% of total energy intake. Lastly, MCT-KD utilize the ketogenic properties of MCT oil to provide half of the required daily fat intake in order to allow for higher intake of carbohydrates and proteins. Though the ketogenic ratio of MCT-KD are similar to that of MAD, recent studies have demonstrated that the ketogenic MCT-oil can be used even without major changes in the dietary patterns.

KD mimic the state of fasting, wherein the body’s supply of glucose is restricted and is thus forced to meet its energetic needs via endogenous production of glucose (gluconeogenesis) and \( \beta \)-oxidation of fatty acids. Since the brain primarily uses glucose as its energy source, the amount of glucose produced via gluconeogenesis will not suffice its needs. Additionally, given that fatty acids cannot cross the blood-brain-barrier, they cannot be utilized as direct source of energy within the brain; thus, the body utilizes alternative strategies to supply the brain’s energy. The increase in fatty acids oxidation within the liver leads to an excessive buildup of acetyl-CoA inside the mitochondrial matrix, which forces the Krebs cycle to produce ketone bodies (KBs) in a process known as ketosis. The three main KBs produced by the liver include \( \beta \)-hydroxybutyrate (BHB), acetoacetate, and acetone, although BHB is the KB that is primarily found in circulation. In the brain, these KB are converted back to acetyl-CoA via their respective enzymes, and can thus re-enter the Krebs cycle to produce ATP.

MCTs are comprised of triacylglycerols of saturated fatty acids mixed with chains that vary in length from 6-10 carbons. They can be synthesized by hydrolysis and subsequent re-esterification of medium-chain fatty acids (MCFA) found in natural resources such as coconut and palm kernel oils. These synthetic MCT oils typically exist as a varying ratio of C8 (caprylic acid) to C10 (capric acid) MCFAs and are classified as safe food products by US Food
and Drug Administration.\textsuperscript{133} MCFA are metabolically and physiologically distinct from long-chain fatty acids (LCFA), as they do not require fatty-acid binding protein or fatty-acid transporters.\textsuperscript{134} Moreover, MCFAs can readily cross the mitochondrial membrane and become oxidized by acyl-CoA synthases.\textsuperscript{134} This will lead to a mitochondrial acetyl-CoA overload, and consequently production of ketone bodies (KB).\textsuperscript{135}

2.2.1 Ketone Bodies: Mechanism of Action

The effects of KB on brain cells have been a topic of interest for many years and has been studied extensively in many neurological conditions.\textsuperscript{131} In the context of PD the main mechanisms through which KBs, particularly BHB, are thought to exert their beneficial effects include: improving brain energy metabolism, moderating neuroinflammation and oxidative stress, and activation of neuroprotective intracellular molecular signaling pathways through G-protein coupled receptors (GPCR) activation and histone deacetylase (HDAC) inhibition.\textsuperscript{107} It is important to note that these mechanisms are not mutually exclusive and that mitigation of deficits in one likely influences the state of the others as well.\textsuperscript{107,132,136,137}

The first, and perhaps the most important effect of BHB on the brain cells is improving brain energetics.\textsuperscript{107} As alluded to before, one of the prominent features of PD is impairments of the mitochondrial complex I, which is needed for efficient energy production.\textsuperscript{35} BHB helps circumvent this deficit in two ways: 1) by increasing the redox potential of the electron transport chain through decreasing the ratio of oxidized nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) to NADH (reduced NAD), while increasing the ratio of oxidized coenzyme Q (Q) to the reduced QH\textsubscript{2} and 2) via bypassing complex I and feeding the electrons into mitochondrial complex II.\textsuperscript{107} The increase in the redox energy following the introduction of BHB, enables more protons to be
pumped into the mitochondrial intermembrane space, thereby increasing the potential to generate ATP.\textsuperscript{107,138}

In one preclinical study investigating these mechanisms in mice that were exposed to MPTP neurotoxin (commonly used to create PD animal models) but were treated with BHB showed evidence of partial protection against structural and functional deficits, mediated through enhancement of oxygen consumption and a decrease in NAD+/NADH ratio.\textsuperscript{139} Specifically, the BHB treated MPTP-exposed mice exhibited reduced SNPC dopaminergic neuron degeneration, greater preservation of striatal dopamine, and fewer occurrences of parkinsonian motor deficits.\textsuperscript{139} Furthermore, it was discovered that selective inhibition of mitochondrial complex II leads to diminishment of this neuroprotective effect, providing support for the compensatory role of this mitochondrial complex.\textsuperscript{139} This compensatory mechanism utilizes succinate as a primary substrate, which is one of the main by-products of BHB catabolism and an oxidative fuel for complex II.\textsuperscript{107}

An alternative hypothesis posits that BHB is able to reverse the inhibition of both mitochondrial complexes I and II.\textsuperscript{140} This was tested in SH-SY5Y dopaminergic neuroblastoma cells treated with either rotenone, a mitochondrial complex I inhibitor, or 3-nitropropionic acid, a mitochondrial complex II inhibitor.\textsuperscript{140} Rotenone treatment caused the loss of mitochondrial membrane potential and the expulsion of cytochrome c content into the cytoplasm while cells treated with 3-nitropropionic acid displayed a lower reducing power.\textsuperscript{140} Nonetheless pre-treatment with or addition of BHB mitigated these deficits similarly.\textsuperscript{140}

Similarly, another study reported that treatment of rotenone-exposed transgenic \textit{Caenorhabditis elegans} (\textit{C. elegans}) PD models with BHB leads to a partial rescue of these nematodes, while
probucol (an antioxidant) and tauroursodeoxycholic acid (an anti-apoptotic bile acid) also exhibited partial protective effects.\textsuperscript{37} Interestingly, the combination of BHB with tauroursodeoxycholic acid but not with probucol, provided complete protection against complex I inhibitors.\textsuperscript{37}

Another way through which BHB can exert its neuroprotective effects is by reducing the production of reactive oxygen species (ROS) and enhancing the antioxidant defensive mechanisms, thereby decreasing the overall oxidative stress of the cell.\textsuperscript{107,131,141,142} In vitro studies have determined that KBs can significantly reduce ROS levels in neurons and mitochondria exposed to hydrogen peroxide.\textsuperscript{141} It has been suggested that the antioxidative effects of KBs are mediated through potassium sensitive ATP channels (K\textsubscript{ATP}) that are localized at the mitochondrial inner membrane and regulate its homeostasis.\textsuperscript{142}

BHB is also a ligand for the G-Protein Coupled Receptor (GPCR) hydroxycarboxylic acid receptor 2 (HCAR2), which involves several important intracellular signaling pathways such as NFκB.\textsuperscript{98,107,137} Interestingly, HCAR2 receptors are found to be upregulated in the substantia nigra microglial cells of patients with PD\textsuperscript{143} and neuroinflammation animal models of PD (induced by nigral injection of LPS).\textsuperscript{144} Coincidentally, treatment of LPS-induced PD rat models with BHB attenuated the resulting neuroinflammation via NFκB-dependant inhibition of proinflammatory cytokines, through the activation of HCAR2 receptors.\textsuperscript{144} BHB-induced activation of HCAR2 leads to inhibition of the NFκB transcription factor\textsuperscript{145}, which normally promotes the expression of neuroinflammatory cytokines such as TNF-\textalpha and IL-1β, and activation of inducible nitric oxide synthase (iNOS), and consequently, generation of NO, which contributes to the oxidative stress of the cell.\textsuperscript{40}
Another potential target for BHB are histone deacetylases (HDACs) such as SIRT-1 which belongs to the family of NAD\(^+\)-dependent deacetylases Sirtuins (SIRTs).\(^{107,145}\) SIRT-1 has been associated with many neuroprotective and life-promoting effects, however it is believed to be downregulated in the brains of patients with PD.\(^{145}\) Furthermore, overexpression of SIRT-1 in cells treated with rotenone has been shown to protect these cells from toxin-induced cell death through mitigation of oxidative stress.\(^{145}\) Intriguingly, it was also discovered that a high-fat diet can prevent premature aging in mice models of Cockayne syndrome (a progressive neurodegenerative disease) through BHB-mediated activation of SIRT-1.\(^{146}\) Additionally, BHB-mediated SIRT-1 activation is also associated with upregulation of autophagy, the process of packaging and recycling of damaged cellular compartments\(^{147}\), activation of PGC-1\(\alpha\), a master regulator of mitochondrial biogenesis\(^{107}\), and activation of transcription factor p53, which has been implicated in promoting cell survival and downregulation of apoptosis\(^{146}\). Another potential anti-apoptotic mechanism of BHB is the inhibition of caspase-3 through increasing the expression ratio of \(Bcl-2/Bax\) genes.\(^{148}\) \(Bcl-2\) is thought to mediate the inhibition of stress-induced cell death and promotion of cell proliferation, while \(Bax\) performs the opposite function.\(^{148}\)

Finally, BHB can regulate gene expression activity by targeting other HDACs.\(^{107,137}\) For instance, BHB-mediated inhibition of HDAC I is associated with upregulation of antioxidant-related genes \(Foxy3\) and \(Mt2\)\(^{149}\) and an increase of the expression of brain-derived neurotrophic factor (BDNF) gene, \(Bdnf\)\(^{150}\), which has shown to be severely depleted in the substantia nigra of PD patients\(^{151}\). Additionally, BHB supplementation has shown to increase the mean lifespan of \(C.\) elegans nematodes by approximately 20% through mechanisms involving HDAC inhibition,
as knocking down HDACs with RNAi prevented BHB from further extending C. elegans’ lifespan.\textsuperscript{152} Moreover, BHB treatment was also associated with a decrease in α-synuclein aggregation and partial improvements in mitochondrial complex I function.\textsuperscript{152}

In summary, KBs (particularly BHB) can target multiple disease mechanisms in patients with PD including amelioration of bioenergetic deficits, neuroinflammation, oxidative stress, and deficits of neuroprotective molecules and proteins. The following section will summarize a number of clinical studies investigating the safety and efficacy of ketogenic interventions in neurodegenerative diseases with a focus on their application in patients with PD.

\subsection{Ketogenic Interventions: Evidence from Clinical Studies}

KD have been used to treat many clinical conditions raging from diabetes and obesity to various cancers and cardiovascular disease.\textsuperscript{153,154} Nonetheless, the oldest application of this diet was to treat neurological conditions, specifically pharmaco-resistant cases of epilepsy.\textsuperscript{155} Recently, the application of KD has expanded to neurodegenerative disorders such as Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), and PD with varying levels of success.\textsuperscript{131} Nonetheless, the difficulties in preparation and unpalatability of KD have fueled the rise of alternative ketogenic interventions such as supplementation with MCT oil or other exogenous sources of KBs.\textsuperscript{127} While there have not been any clinical trials investigating the effects of MCT oil on patients with PD, three clinical trials have been conducted on the use of KDs in PD.\textsuperscript{99–101} The following sections will examine these studies and review the evidence on MCT oils using a combination of preclinical studies in PD animal models and clinical studies in non-PD cohorts.

\subsection{Ketogenic Diet Studies}
The first clinical trial to examine the feasibility of KD on patients with PD was conducted by VanItallie et al., in 2005. This study was done with 7 participants (with 5 completing the study) who adhered to a strict 4:1 KD (90% fat, 8% protein, 2% carbohydrates) for 28 days. The mean serum KB concentration in the three most adherent participants was reported as 6.6 mmol/L, which was strongly correlated with daily urine ketone measurements. It must be noted that the typical KBs concentration due to nutritional ketosis ranges from 0.5 mmol/L to 6 mmol/L measured via blood BHB levels, though higher measures have also been reported with long-term starvation. This is remarkably different from blood KB concentration of ketoacidosis, a pathological condition associated with diabetes mellitus, which is in the range of 20-25 mmol/L. All five participants displayed signs of symptomatic improvements as determined by their total UPDRS scores, however, the most noticeable improvements were observed in resting tremors, freezing, balance, gait, mood, and energy levels. Although this trial was not adequately powered to make any claims regarding the efficacy of KD in patients with PD, it paved the way for future larger and more comprehensive trials.

The study, by Phillips et al., investigated the KD in 47 (with 38 finishing the study) patients with PD. Participants were randomized into either a KD or a low-fat high carbohydrate (LFHC) diet group and adhered to their respective diet for 8 weeks. The KD in this study was much less restrictive with fats composing about 60% of total daily energy intake. In addition to changes from baseline in the UPDRS score, this study also analyzed several metabolic parameters including changes in the participants’ lipid profile, urate, and C-reactive protein. The two groups differed significantly in the blood glucose and serum BHB levels with the ketogenic group having lower glucose (weekly mean = 5.7 mmol/L in KD group vs. 6.28 in the low-fat group) and higher BHB concentration (weekly mean = 1.15 mmol/L in KD group vs.
0.16 mmol/L in the low fat group) levels. Both diets resulted in improvement in motor and non-motor symptoms, however, the non-motor improvements in the KD group were more substantial. Urinary problems, pain, fatigue, daytime sleepiness, and cognitive impairments were the most improved non-motor symptoms in the KD group.

Of note, the KD resulted in an increase in the levels of high- and low-density lipoproteins (HDL and LDL respectively) and cholesterol while the low-fat diet was associated with the opposite effect. After the 8-week intervention, HDL levels increased from 1.5 mmol/L to 1.89 mmol/L, LDL increased from 2.73 mmol/L to 3.42 mmol/L, and total cholesterol levels increased from 5.03 mmol/L to 5.98 mmol/L, in the KD group. There was no difference in the triglyceride levels in any of the experimental groups. Though these alterations in the lipid profile of participants in the KD group may seem undesirable form a cardiovascular standpoint, no such complication was reported in this study. Moreover, higher levels of cholesterol and LDL have been associated with a lower risk of Parkinson’s disease. It is suggested that this protective effect (at least with respect to cholesterol) is in part due to an increase in coenzyme Q10, which has antioxidative properties. If true, this would support a KB-independent mechanism through which KDs can impart their putative benefits.

The third and latest but also very small clinical trial, by Krikorian et al., compared the effects of a KD with a high-carbohydrate western-typical diets for eight weeks in 14 patients with PD-associated mild cognitive impairment (MCI). The KD prescribed in this study restricted carbohydrate intake to 20 g per day without any manipulation of the fat or protein intake. Cognitive performance, motor function, and metabolic parameters were analyzed before and after 8 weeks of adherence to the respective diet. In the KD group the mean BHB levels
reached 0.31 mmol/L, which was significantly higher than baseline value of 0.08 mmol/L, measured via point of care blood samples. There were no differences in the fasting glucose levels, however, insulin levels decreased significantly in both dietary groups. Though motor function was not changed with either dietary intervention, cognitive performance on the lexical access composite (the Controlled Oral Word Association task) and the memory composite (Verbal Associate Learning Test), were significantly improved in the KD group. These reflect improvements in the executive control and memory encoding domains, respectively. Though no detailed analysis of adverse events was reported in this study, one participant reportedly discontinued intervention due to an unspecified perceived side effect.

The variability in the KD regimens utilized in these studies and their small sample size makes it difficult to draw any conclusions regarding the feasibility or the efficacy of KD in patients with PD. Furthermore, difficulties with blinding and having a true control diet are also inherent to trials investigating dietary intervention, which further complicates the interpretation of results, particularly those that may be affected by the placebo effect. Lastly, monitoring adherence to a given dietary intervention is another challenge faced by dietary clinical trials. Studies often employ a multi-pronged monitoring system that assess food intake via diet journals and biological parameters such as blood BHB levels to determine the overall adherence of the diet, however, these measures often rely on participants self-reports, as well as increasing the burden of participation. Nonetheless, these studies do provide some promising preliminary evidence regarding the potential of ketogenic interventions in ameliorating PD motor and non-motor symptoms. Table 2.2 summarizes the findings of these studies.
<table>
<thead>
<tr>
<th>Study Design</th>
<th>Dietary Intervention</th>
<th>Main Findings</th>
<th>Limitations</th>
<th>Author</th>
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<tr>
<td>Open-label feasibility</td>
<td>Classic KD with a 4:1 ratio (n=7; 5 completed) for 28 days</td>
<td>blood BHB levels increased with the diet. Improvements in UPDRS scores were</td>
<td>Very small sample size. Lipid profile was not reported on in sufficient</td>
<td>VanItallie et al.²⁹⁹</td>
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<td></td>
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<td>observed.</td>
<td>details. The PD symptoms analysis was not sufficiently comprehensive. There</td>
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<td>was no investigation into the diet’s mechanism of action.</td>
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<tr>
<td>Randomized Controlled</td>
<td>KD with a 3:1 ratio (n=24; 18 completed) versus a low-fat diet (n=23; 20 completed)</td>
<td>Higher blood BHB and lower blood glucose levels in the KD group vs. low-fat</td>
<td>No investigation into the diet’s mechanism of action was conducted. A more</td>
<td>Phillips et al.¹⁰¹</td>
</tr>
<tr>
<td>Trial</td>
<td></td>
<td>diet group. Greater improvement of non-motor symptoms in the KD group vs. the</td>
<td>comprehensive analysis of PD symptomology using specific validated</td>
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<td></td>
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<td>low-fat diet group.</td>
<td>questionnaires can better elucidate the diet-induced symptomatic changes.</td>
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<td></td>
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<td>Additionally, a more comprehensive analysis of biological markers can</td>
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<td>provide a mechanistic explanation for the observed symptomatic changes.</td>
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<tr>
<td>Randomized Controlled</td>
<td>Low-carbohydrate KD (n=7) vs. Western-typical diet (n=7) for 8 weeks.</td>
<td>Significant improvement of cognitive function in the KD group as compared to</td>
<td>Very small sample size. Ketosis was achieved using carbohydrate restriction</td>
<td>Krikorian et al.¹⁰⁰</td>
</tr>
<tr>
<td>Trial</td>
<td></td>
<td>the control diet.</td>
<td>alone, thus the KB levels are relatively lower than other studies. No</td>
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<td></td>
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<td>investigation into the diet’s mechanism of action was conducted. The</td>
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<td></td>
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<td>relationship between plasma BHB levels and cognitive performance was not</td>
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<td></td>
<td>explicitly reported on.</td>
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**Table 2.2** Clinical studies of KD in patients with PD
Despite their clinical promise, there are several issues and challenges associated with these dietary regimens that limits their widespread applicability. First, the preparation and long-term adherence to KD has proved to be quite challenging, particularly in those with chronic neurodegenerative conditions, as these diets are typically perceived as unpalatable and requiring drastic changes in the adherents’ lifestyle. Second, these diets are associated with significant weight loss, which is already a problematic comorbidity related to gastrointestinal dysfunction in PD. Lastly, prolonged intake of fatty foods has been associated with gut dysbiosis and consequently, aggravation of various pathophysiological mechanisms in PD such as gut barrier hyperpermeability and inflammation (the relationship between KD and PD gut dysbiosis is reviewed in more details under section 2.3.3). Hence, it has been proposed that a more moderate increase in blood ketone levels through supplementation with medium-chain triglycerides (MCTs) on the background of a less restrictive diet may be a safer and more realistic alternative to the strict KD.

2.2.2.2 MCT Oil Studies

MCT oil supplementation has not been clinically trialed in patients with PD. In one preclinical study MCT oil was used to provide 50% of energy to mice models of PD who were otherwise fed a regular diet. This study reported significant elevation of blood BHB levels (from 0.25 mmol/L to 1.83 mmol/L) following treatment with MCT which was associated with better motor performance in MCT-treated rats as compared to controls. Nonetheless, the proportion of total energy obtained from MCT oil as compared to other food ingredients in this study is simply not feasible in humans; thus, extrapolation of these results to individuals with PD would be
unrealistic. As such, clinical trials done in non-PD cohorts may provide better evidence in the potential utility of MCT treatment for patients with PD.

Several randomized clinical trials have reported significant success using MCTs to improve cognitive performance in patients with Alzheimer’s disease (AD) and Mild Cognitive Impairments (MCI). For instance, in one of the first studies investigating the effects of MCT treatment in 20 patients with AD and MCI, the participants were given either emulsified MCT (40 ml) or placebo on two separate occasions and their cognitive performance was assessed using the Alzheimer’s Disease Assessment Scale – Cognitive Subscale (ADAS-Cog). Both the MCT drink and the placebo (long-chain triglycerides) provided 690 calories of energy. The plasma BHB levels were significantly elevated from 0.04 mmol/L to 0.54 mmol/L 90 minutes after the intake of MCT, which corresponded with a significant improvement in cognitive performance on paragraph recall tests as compared with the placebo treatment. Moreover, this effect was moderated by the APOε4 genotype with improvements in cognitive performance being observed in ε4- participants and not in those with an ε4+ genotype. These results were replicated by a more recent study in China, who also determined that treatment with three daily doses of MCT oil (equivalent to 17.3g per day) was associated with improvements in cognitive performance as measured by ADAS-Cog relative to treatment with a placebo. Interestingly, although the increase from baseline in blood BHB levels was significant in this study (from 0.039 mmol/L to 0.09 mmol/L), the magnitude of this increase was not comparable to that of the previously mentioned study.

Another randomized controlled trial investigated the efficacy of an MCT-based ketogenic drink in individuals with MCI via a two-phased study. During the both phases, the interventions consisted of two daily supplemental drinks with an equivalent dose of 30g of MCT per day for 6
months with.\textsuperscript{130} In the first phase, PET scans were utilized to analyze the brain metabolism of ketones and glucose in 52 (n=39 finished the intervention; 19 in the MCT group vs. 20 in the placebo group) individuals with MCI in response to treatment with MCT drink versus a placebo.\textsuperscript{130} This study reported that those who received the MCT drink displayed a 230% increase in brain metabolism of ketones with no change in glucose uptake.\textsuperscript{130} Moreover, this change was positively correlated with improvements in cognitive performance in multiple domains.

During phase 2, the sample size was increased to n = 39 in the MCT group and n = 43 in the placebo group and PET scans were replaced with blood-based metabolic measures. Consumption of the ketogenic drink was associated with a significant elevation in blood BHB (0.149 mmol/L before interventions to 0.401 mmol/L after intervention) and total ketone (0.241 mmol/l before interventions to 0.606 mmol/l after intervention) levels and significant improvements across various cognitive domains (Free and cued recall, verbal fluency, Boston naming test, and Trail-making test) as compared to those in the placebo group.\textsuperscript{129} Furthermore, higher plasma ketone levels were correlated with greater cognitive improvements, at least in some of the tested domains.\textsuperscript{129} Importantly, both these studies lasted for 6 months with two MCT drinks per day with no serious adverse events. The most common adverse event was gastrointestinal discomfort, 50% of which resolved after the first month of treatment.\textsuperscript{129}

Similar results have been produced in the other studies examining the efficacy of MCT oil in improving cognitive performance in patients with AD and MCI, associated with an increase in blood BHB level.\textsuperscript{128,164,165}
Interestingly, another study done in a cohort of healthy older (n = 65; n = 33 in the intervention group) adults reported no cognitive improvements after 3 months of daily supplementation with 18 g of MCT (consumed as three 6 g MCT jelly sticks), however, they observed improvements in walking balance, which was associated with suppression of brain glucose metabolism in the right sensory motor cortex and increased ipsilateral functional connectivity as compared to controls.\textsuperscript{166} It would be interesting to investigate any potential improvement in gate stability of patients with PD following supplementation with MCT oil. Additionally, a meta-analysis of changes in blood triglyceride and lipid profiles with respect to MCT oil has determined that it does not affect total cholesterol, LDL, or HDL levels, though it increases the amount of triglycerides, albeit only slightly.\textsuperscript{167}

\subsection*{2.2.3 Ketogenic Interventions and the Gut Microbiome}

As the importance of gut microbiome in promoting health and its role in various disease states is being uncovered, the interactions between dietary interventions, particularly KDs, and gut microbiota are being considered more seriously.\textsuperscript{160} This is of particular importance in patients with PD, as gut dysbiosis is a well-established feature of the disease \textsuperscript{57,63,68,69}, however, no study to date has investigated this relationship in patients with PD.

The limited number of studies that have investigated the interactions between KD and the gut microbiome suggest a bidirectional relationship wherein KDs influence the bacterial and metabolic composition of the gut\textsuperscript{160,168-170}, yet the gut microbiome is necessary for KDs to confer their benefits.\textsuperscript{91} For instance, it has been shown that mice with a conventionally colonized gut microbiota had reduced susceptibility and incidence of seizures when fed a KD, but not when fed a control gavage.\textsuperscript{91} However, KD was not effective in reducing seizure activity when fed to
germ-free mice, suggesting that gut microbiome plays an important role in conferring the anticonvulsive effects of KD. Furthermore, re-colonization of the gut followed by a KD regimen resulted in reduction of seizure activity in the germ free mice, thereby drawing a causal link between KD’s anticonvulsive properties and the gut microbiome.

The most consistently reported KD-induced changes in the gut microbiota include a decrease in α- and β-diversity, and an increase in the relative abundance of *Akkermansia muciniphila*. The KD-associated reduction in the bacterial diversity has been attributed to the low intake of complex carbohydrates, which are an important energy source for the gut bacteria. In one study, the “anti-microbial” effects of KD has been associated with behavioral improvements in a murine model of Autism Spectrum Disorder. In another study investigating the role of gut microbiome in mediating the anti-seizure effects of KD it was discovered that the relative abundance of *Akkermansia* bloomed from 2.8% to 36.3% in only 4 days and remained elevated throughout the rest of the 14-day intervention. Interestingly, this bloom was determined to be necessary for conferring the anti-seizure effects of the diet. Although a reduction in bacterial diversity is not commonly reported in patients with PD, an increase in the relative abundance of *Akkermansia* has been a consistent finding. Though *Akkermansia* is widely regarded as a beneficial bacteria, in patients with PD higher *Akkermansia* levels have been associated with features of gut dysbiosis such as an increase in gut permeability, particularly in the absence of sufficient dietary fiber.

On the other hand, other studies suggest that KD has the potential to reduce the relative abundance of the genera *Bifidobacterium* and *Lactobacillus*, which are reportedly elevated in patients with PD. For instance, mice fed a KD were found to have lower relative abundance
of *Bifidobacterium* and *Lactobacillus* as compared to mice fed a high-fat (HF) or a high-carbohydrate (HC) diet.\textsuperscript{168} Higher abundance of *Bifidobacterium* in this study was associated with an increase in activation of intestinal Th17 immune cells and consequently, induction of an inflammatory response.\textsuperscript{168} Furthermore, administration of ketone esters to mice fed a HF diet successfully reduced the levels of *Bifidobacterium* and decreased the activation of Th17 cell, thereby reducing intestinal inflammation.\textsuperscript{168}

A potential problem of classic KD with respect to PD is the depletion of SCFA-producing bacteria due to the elimination of fibrous carbohydrates from the diet.\textsuperscript{169} One study examining this effect has reported a substantial decrease in fecal SCFA levels following one month of treatment with a classic KD in patients with epilepsy.\textsuperscript{169} SCFA are crucially important for maintaining gut health as their depletion has been associated with impaired intestinal barrier integrity and release of pro-inflammatory substances (e.g. LPS) into the systemic circulation.\textsuperscript{76,77} Furthermore, lower fecal SCFA levels and SCFA-producing bacteria has been commonly and consistently reported in patients with PD.\textsuperscript{56,69,80}

Notwithstanding, a recent study examining the effects of KD on children with drug refractory epilepsy has found that although all major SCFAs were found at significantly lower levels in the epileptic cohort as compared to healthy controls at baseline, there was no significant difference between the two groups after 6 months of intervention.\textsuperscript{175} Even though stool SCFA levels were not significantly altered after the KD intervention, there was a trend towards an increase in the epileptic cohort.\textsuperscript{175} Additionally, the bacterial species that were found at elevated levels at baseline, including *Bifidobacterium* and *Akkermansia* were significantly reduced after six months of KD.\textsuperscript{175}
Similar to SCFAs, the evidence regarding the effects of KD on gut-mediated inflammation is also contradictory and heterogeneous. For instance, in one study KD was used to successfully treat colitis in dextran sulfate sodium (DSS)-treated mice.\textsuperscript{176} The beneficial effects of KD in this study were attributed to the changes it induced in the gut microbiome, including a reproducible increase in Akkermansia, as transplantation of the KD-fed microbiome into germ-free mice also provided protection against DSS-induced colitis.\textsuperscript{176} On the other hand, a similar study using the same treatment observed an aggravation of the colitis symptoms, which was associated with impairments of gut barrier integrity and increased intestinal and systemic inflammation markers.\textsuperscript{177} Another study found that a 3-month intervention with classic KD to be associated with a significant increase in the sulfate-reducing bacteria \textit{Desulfovibrio} in the large intestine, which has the potential to aggregate intestinal inflammation.\textsuperscript{178} The increase in \textit{Desulfovibrio} is thought to be driven by high consumption of animal-based fats.\textsuperscript{178}

Additionally, the KD-gut microbiome interactions are further complicated when the underlying environmental conditions are considered as well.\textsuperscript{160} For instance, it has been shown that KD exacerbates the cognitive impairment induced by hypoxia in mice, yet the diet alone is not sufficient to deteriorate spatial learning and memory performance.\textsuperscript{179} Notably, this exacerbation was found to be driven by KD-induced alterations in the gut microbiome, particularly an increase in the relative abundance of \textit{Bilophila wadsworthia}, as transferring the microbiome of the KD-fed mice into mice fed a control diet also led to cognitive impairments.\textsuperscript{179}

At any rate, based on the evidence presented above, it is obvious that the effects of KD on the gut microbiome are complex and dependent on many factors. To this end, a few studies have investigated a modified Mediterranean-Ketogenic diet (MeDi-KD) as a more balanced
alternative to the conventional KD in patients with MCI. MeDi-KD combine the principles of MeDi and KD such that the intake of dietary fibers are generally increased, while the intake of animal-based fats are limited. Furthermore, there is greater allowance for the intake of greens and vegetables as compared to conventional KD, however, fats are still the major source of energy (~60-65% of daily caloric intake). Analysis of the gut microbiome following adherence to a MeDi-KD shows an increase in SCFAs butyrate and propionate, and a reduction in acetate and lactate. Furthermore, a decrease in Bifidobacterium levels was also reported in participants with MCI and in the MeDi-KD group. Additionally, participants on the MeDi-KD displayed an increase in cerebral perfusion and KB uptake levels, without affecting glucose metabolism, when compared to the low-fat control diet (American Heart Association Diet).

2.3 Conclusion

The evidence presented here shows the potential for dietary interventions as therapeutic avenues for treating PD. While preliminary studies have reported promising clinical outcomes with both MeDi and KD, further research is warranted to determined their safety, feasibility, and ultimately, the efficacy of these diets on patients with PD. Furthermore, MeDi-KD have the potential to integrate the beneficial aspects of both these diets, while minimizing the KD-associated gut-related risks, and are thus a great candidate for application in patients with PD. The next chapter outlines a protocol for a clinical trial investigating the safety and feasibility of MeDi-KD and Mediterranean diet supplemented with MCT oil in people with PD.
Chapter 3: The Safety and Feasibility of Mediterranean-Ketogenic Dietary Interventions on Gut Health in Parkinson’s Disease: A Protocol for an Open-label, Randomized, Crossover Design Clinical Trial (KIM Trial)

3.1 Introduction

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

Emerging evidence suggests that both ketogenic diet (KD)\textsuperscript{98–100,127,184,185} and Mediterranean (MeDi) diet\textsuperscript{102,103,110,113,122,123} have beneficial and likely complementary effects in PD. Combining principles from both diets would likely optimize dietary benefits. To the best of our knowledge, no clinical trials have yet been performed into the combined KD and MeDi-style dietary interventions in PD.

MeDi diets are primarily but not exclusively plant-based\textsuperscript{113}. Their promotion of high fiber content intake promotes the production of SCFA and are associated with improved gut microbiome diversity, reduced oxidative stress, and improved insulin sensitivity\textsuperscript{110}. Adherence to MeDi in PD is associated with a reduced risk of developing parkinsonism or prodromal PD\textsuperscript{102,103,122,123} and a higher age of PD onset\textsuperscript{105}.

KD\textsuperscript{s} are high in fat, adequate in protein and very low in carbohydrates\textsuperscript{186}. Unlike the MeDi, KD stimulates the synthesis of ketone bodies (KB) that are used as an alternative fuel source to glucose\textsuperscript{187}, the utilization of which is perturbed in the PD brain\textsuperscript{107}. Fatty acids are converted to KB\textsuperscript{s} such as beta-hydroxybutyrate (BHB)\textsuperscript{107,186–189}. BHB, in turn, is used for mitochondrial ATP generation\textsuperscript{187} through bypassing the deficient complex I activity in the PD mitochondria\textsuperscript{107}. Additionally, BHB also exhibits anti-oxidative\textsuperscript{149} and anti-inflammatory properties\textsuperscript{190} (review by\textsuperscript{137}).

KD\textsuperscript{s} have been successfully used for decades to treat pharmaco-resistant cases of epilepsy\textsuperscript{155}, and more recently, in diabetes\textsuperscript{153}, obesity\textsuperscript{154}, and neurological disorders\textsuperscript{127,185,191}. Small scale pilot trials in PD report improved Movement Disorder Society – Unified Parkinson’s Disease
Rating Scale (MDS-UPDRS) scores\textsuperscript{99}, cognitive performance \textsuperscript{100} and non-motor symptoms \textsuperscript{184} (recent review by \textsuperscript{98}).

Another method for inducing serum ketone levels is by consumption of ketogenic medium-chain triglycerides (MCTs; 8-carbon tricaprylin, 10-carbon tricaprin) \textsuperscript{133}. MCTs are converted to KBs, which can readily cross the blood-brain barrier and the mitochondrial membrane and be utilized as an efficient energy source \textsuperscript{133}. MCTs can elevate the blood KB levels without the need for carbohydrate intake restrictions or calory deficiency, however, the body will not endogenously produce KBs (i.e., enter the state of ketosis) in the absence of the aforementioned restrictions \textsuperscript{133}. Therefore, MCT oil supplementation yields a milder elevation in KBs than the KD, however, the ketogenic benefits appear to be largely preserved as shown in studies of epilepsy \textsuperscript{192} and cognitive impairment \textsuperscript{129,130}. For instance, it has been shown that consumption of MCT supplements can increase brain ketone metabolism by 230\% in patients with mild cognitive impairment accompanied by significant cognitive improvements \textsuperscript{129,130}.

Studies of classical KDs in other neurological conditions have reported significant alterations within the gut microbiome, including an increase in \textit{Akkermansia} \textsuperscript{160}. For instance, it has been reported that classical ketogenic diets result in a significant increase of \textit{Akkermansia} in mice models of epilepsy \textsuperscript{193}. \textit{Akkermansia} plays a critical role in the anti-seizure effect of the KD, which blooms by more than 30\% within days of starting the diet \textsuperscript{193}, however, given the aforementioned increased levels of \textit{Akkermansia} in PD, a further increase might be detrimental. The classic KD can also reduce fecal SCFA levels, which are important in promoting gut health \textsuperscript{169}.
By combining the principles of MeDi diet with KD, we hope to leverage the gut-health promoting aspects of the former with bioenergetics benefits of the later in a safe manner.

3.2 Methods

3.2.1 Overview and Aims

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

The primary aim of this trial is to examine the safety of MeDi-KD and MeDi-MCT interventions with respect to gut health focusing on SCFA production. Specifically, the primary outcome measure with respect to this aim is changes from baseline in fecal butyrate levels as reduced SCFA and butyrate levels are key findings in PD \(^{24,56-59}\) and can lead to increased systemic and neuronal inflammation \(^{57}\). Although the immunomodulatory effects of SCFA’s are complex with both pro- and anti-inflammatory functions \(^{116,181,194}\), reduced faecal butyrate levels are best established as detrimental in PD, correlating with age of onset and inversely correlating with motor and non-motor symptoms in PD \(^5\).

Other measures of gut health – such as changes in measures of gut inflammation (using fecal and blood calprotectin as the biomarker), gut-barrier integrity (using fecal and blood zonulin as the biomarker), and the taxonomic composition of gut microbiome before and after each 8-week intervention \(^{84,86,195}\) – will be also assessed as secondary outcome variables.

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

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

3.2.2 Study Design

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

3.2.2.1 Recruitment Procedures

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

### 3.2.2.2 Consent and Screening

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

Following the obtainment of an informed consent, the participants will complete the screening assessments, including the Montreal cognitive assessment (MoCA), the Beck Depression Inventory-II (BDI-II), the Movement Disorder Society-Unified Parkinson’s Disease Rating Scale (MDS-UPDRS), as well as a past medical history and medication review. **Table 3.1** outlines all the inclusion and exclusion criteria for this trial. Individuals must meet all eligibility criteria in order to participate in the study. There is no limitation on concomitant medication while taking the MCT supplement, however, we include the use of probiotic in the last 4 weeks and antibiotics in the last 3 months prior to the trial, and immunomodulatory agents as exclusion criteria.
### Inclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tbody>
<tr>
<td>Age between 40-85 years</td>
<td>Atypical parkinsonism</td>
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<tr>
<td>PD diagnosis based on Movement Disorder Society (MDS) criteria</td>
<td>Medical or psychiatric conditions that would prevent full participation in the nutrition intervention</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr stage between 1 to 3</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>On stable dopaminergic medications for at least one month</td>
<td>Significant dysphagia</td>
</tr>
</tbody>
</table>

- Diabetes on insulin
- Anti-coagulation on warfarin
- Inflammatory bowel disease
- Dementia defined by a Montreal Cognitive Assessment (MoCA) score of less than 21
- Inability to fill in electronic questionnaires or understand study instructions
- Use of immunomodulatory agents
- Probiotic use in the last 4 weeks (except for dietary sources such as yoghurt, kefir etc.), or antibiotic use in the last 3 months prior to the trial
- Use of MCT oil or on KD in last 8 weeks prior to the trial
- Allergic to MCT oil, coconut oil, or coconut

**Table 3.1** Inclusion and exclusion criteria for KIM clinical trial.

**3.2.2.3 Study visits**

**Figure 3.1** summarizes all the events, intervention, and assessments and the timepoints at which they will occur during the duration of the study.
<table>
<thead>
<tr>
<th></th>
<th>Pre-Enrolment</th>
<th>First Intervention Period</th>
<th>Washout</th>
<th>Second Intervention Period</th>
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<tbody>
<tr>
<td></td>
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<td>Pre-intervention 1 (visit 1)</td>
<td>Post-intervention 1 (visit 2)</td>
<td>Washout check-in</td>
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<td>MeDi-MCT</td>
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<td>Participant Health Assessment</td>
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<tr>
<td>Demographic Information (sex, age, year of diagnosis, age of onset, initial symptoms)</td>
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<td>Medication overview</td>
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<td>Past Medical History</td>
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<tr>
<td>Weight</td>
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<td></td>
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<tr>
<td>Height</td>
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<td>X</td>
<td></td>
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<tr>
<td>Waist Circumference</td>
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<tr>
<td>Other Participant Information</td>
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<tr>
<td>Family History/Ancestry</td>
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<td>Alcohol Use/Smoking</td>
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<tr>
<td>Gynecological History</td>
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<tr>
<td>Montreal Cognitive Assessment (MoCA)</td>
<td>X&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Movement Disorder Society - Unified Parkinson’s Disease Rating Scale (MDS-UPDRS)</td>
<td>X&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td>X</td>
<td>X</td>
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<td>Fatigue Severity Scale (FSS)</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Parkinson Anxiety Scale (PAS)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Starkstein Apathy Scale (AS)</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Beck Depression Inventory, 2nd Ed. (BDI-II)</td>
<td>X&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
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<td>X</td>
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<sup>*</sup> Indicates the presence of specific data points.
<table>
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<tr>
<th>Assessment</th>
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<th>Washout</th>
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</tr>
</thead>
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<tr>
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<td>Pre-intervention 1 (visit 1)</td>
<td>Post-intervention 1 (visit 2)</td>
<td>Washout check-in</td>
</tr>
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<td>Parkinson’s Disease Questionnaire – 39 (PDQ-39)</td>
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<td>ROME III Constipation Module (ROME III)</td>
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<td>X</td>
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<td>Diet History Questionnaire II (DHQ-II)</td>
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<td>Bristol Stool Chart</td>
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<tr>
<td>National Health Institute Toolbox Cognitive Battery (NIHTB-CB)</td>
<td></td>
<td>X</td>
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<td>X</td>
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<td>Patient Global impression of Change (PGIC)</td>
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<td>WHO Quality of Life Questionnaire</td>
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<td>Fecal Sample</td>
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<tr>
<td>Qualitative Interview</td>
<td></td>
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</table>

**Figure 3.1** KIM clinical trial summary of study visits.

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

In short, if individuals continue to meet the eligibility criteria, they will commence the first preintervention visit (visit 1) wherein a study team member will extract and collect de-identified clinical and demographic data including past medical history, disease age of onset, initial PD
symptoms, medications, and anthropometric measurements such as height, weight, and waist circumference. Additionally, a series of questionnaires and assessments measuring the participants’ motor and non-motor clinical symptoms and their lifestyle and dietary habits will be administered. Table 3.2 outlines all the questionnaires that will be administered and their respective purpose.

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>MDS-UPDRS</td>
<td>PD severity 200</td>
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<td>Fatigue Severity Scale</td>
<td>Measuring Fatigue in patients with PD 201</td>
</tr>
<tr>
<td>Starkstein Apathy Scale</td>
<td>Measuring Apathy in patients with PD 202</td>
</tr>
<tr>
<td>BDI-II</td>
<td>Assessing symptoms of depression 203</td>
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<td>Parkinson Anxiety Scale</td>
<td>Assessing anxiety in patients with PD 204</td>
</tr>
<tr>
<td>Parkinson’s Disease Questionnaire-39</td>
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<tr>
<td>Physical Activity Scale for Individuals with Physical Disabilities</td>
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<tr>
<td>Bristol Stool Scale</td>
<td>Assessing stool consistency 207</td>
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<tr>
<td>Rome III</td>
<td>Constipation and irritable bowel symptoms 208</td>
</tr>
<tr>
<td>Patient Global Impression of Change</td>
<td>Assessing perception of change due to the interventions</td>
</tr>
<tr>
<td>World Health Organization quality of life questionnaire</td>
<td>General quality of life</td>
</tr>
<tr>
<td>The Canadian version of the Diet History questionnaire</td>
<td>Dietary habits prior to beginning of the study 209</td>
</tr>
<tr>
<td>The National Heath Institute Toolbox</td>
<td>Assessment of cognitive function 210</td>
</tr>
<tr>
<td>Cognitive battery</td>
<td></td>
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</tbody>
</table>

Table 3.2 KIM trial clinical questionnaires
Subsequently, a qualified study team member will collect two tubes of blood (a total of 12 mL) from the participants by venipuncture with an evacuated tube system. Blood collection will occur at the PPRC in the UBC Movement Disorders Clinic. Proper sanitary and disinfection protocols will be followed stringently.

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

Following the completion of the initial assessments and the blood sample collection, the participants will be randomly allocated to start with either the MeDi-KD or the MeDi-MCT with a 1:1 ratio, using variable block sizes. Randomization will be computer-generated (e.g., using the Blockrand package in R) and conducted by an independent statistician prior to the enrollment of the first participant. Subsequently, MCT oil supplies will be provided to those randomized into the MeDi-MCT intervention arm. Relevant instructions on the dosage will be provided by the registered dietitian. Moreover, all participants will be provided with a study journal, which they will use to track their daily intake of the MCT oil supplement (those in the MeDi-MCT arm), bowel movements, pseudorandom one-day summary of their food intake, and any adverse reactions. The procedures described above (except for the randomization and administration of the DHQ questionnaire) will be repeated during the subsequent pre-intervention visit (visit 3) prior to commencing the second intervention phase.
Following the completion of the pre-intervention visit, the participants will spend the next 8 weeks in the respective intervention arm (see Interventions below for more information). Phone check-ins will be completed at the week 4 mark of each intervention phase during which compliance and adverse events will be queried (Participants will be provided with the relevant contact information of the research team and asked to report adverse events at any time during the duration of the study). Additionally, at each check-in, the participants will be asked to remotely complete a number of questionnaires assessing their general quality of life.

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

After completing the first 8-week intervention, the participants will start the 8-week washout period, where they will return to the same lifestyle and dietary habits they were following prior to the start of the study. We will capture baseline dietary habits with the food frequency questionnaire at the first visit. Similar phone check-ins will be conducted at week 4 of the washout period. There will be no in-person visits scheduled for the washout period.
changes lead to gut microbiome changes within days \(^{211}\), therefore, the 8-week washout period should be sufficient to avoid any carry-over effects.

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

### 3.2.3 Interventions

#### 3.2.3.1 MeDi-KD

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

MeDi-KD is not associated with any significant risks \(^{180,196}\). Contrary to the classical KDs that mainly rely on animal-based fats \(^{212}\), the MeDi-KDs utilize plant-based fats as the primary source of calories. High consumption of animal-based fats can be associated with an increased risk of developing coronary heart disease \(^{127}\), however the plant-based fat sources used in the MeDi-KD
have been shown to reduce the risk of hypercholesterolemia and other blood lipid abnormalities.

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

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

3.2.3.2 *MeDi-MCT*

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

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

1) The dose is not tolerable.

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

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

The most common associated side effect of MCT oil is gastrointestinal (GI) upset, which are typically mild and transient in nature and can be treated with over-the-counter medication and stopping the supplement. The dose titration during the first week should minimize the risk of developing GI symptoms, however, we will monitor the participants for such symptoms throughout the intervention period. Previous studies have determined that 50 g of MCT per day is safe and well tolerated by the participants 213.

### 3.2.4 Assessment of Compliance

Dietary adherence will be fostered with a multi-pronged strategy following manuals and standard operating procedures similar to prior large scale, successful dietary interventions 122,211. All participants will have two individual sessions with an RD in the first two weeks of each intervention, and weekly check-ins for the remainder of each intervention thereafter. To ensure
adherence to the protocol and to successfully address barriers and challenges, individual sessions are standardized but will allow for individualization depending on the needs of the participants.

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

Additionally, participants will also be asked to fill in a 3-day food record at the beginning of the intervention (capturing two weekdays and a weekend day) and pseudo-random one-day records at weekly intervals to ensure adherence and successful implementation of the respective protocol. Moreover, blood ketone body levels will be self-monitored by the participants at home once weekly using the commercially available Freestyle Precision (Abbott) device and Freestyle Precision blood ketone strips. The participants will be instructed to insert a test strip into the monitor, use a lancing device (Freestyle Lancing Device II and lancets) to prick their fingers and obtain a small drop of blood, and place the blood drop on the test strip. The measurements will be stored in the device until the participants’ post-intervention study visit, where the study team members will extract the data. Previous studies have found blood BHB levels to be more accurate in predicting benefits of ketosis as compared to daily urine ketone monitoring, even if blood analyses are done less frequently\textsuperscript{214,215}.

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

3.2.5 Data Analysis

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

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

DNA extraction, library preparation, sequencing, and processing will follow locally established procedures. In short, DNA is extracted with a KingFisher robot using the Qiagen MagAttract PowerSoil DNA KF kit (Formerly MO Bio PowerSoil DNA Kit). Following quality control, libraries are prepared with the Illumina Nextera library preparation kit (Illumina, San Diego, CA, USA). Pair-end sequencing (150 bp x 2) is done on an Illumina NextSeq 500.

Shotgun metagenomic sequence reads are processed with the Sunbeam pipeline. Processing includes adapter removal, read trimming, low-complexity-reads removal, and host-sequence removals with referencing to the Genome Reference Consortium Human Reference 37. The remaining reads are taxonomically classified with the MiniKraken2_v1 database. For functional profiling, high-quality (filtered) reads are aligned against the SEED database via translated homology search and annotated to Subsystems, or functional levels, 1-3 using Super-Focus.
**Fecal SCFA content:** SCFA analysis (including butyrate, acetate, propionate) will be carried out in the freshly frozen faecal samples, using a well-established gas chromatography-mass spectrometry (GC-MS) protocol. In brief, 1 mL of ice-cold 10% isobutanol will be added to 100-150 mg of fecal sample to extract SCFAs from fecal samples. The supernatant will then be processed and labeled by isobutyl chloroformate. The labeled SCFA samples will be loaded on an Agilent GC-MS system for SCFA analysis.

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

### 3.2.5.2 Blood Sampling and Analysis

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

The blood samples will be analyzed for the following:

**KBs:** In addition to the continuous monitoring of Blood BHB levels throughout the intervention period, we will also measure blood KB levels using the blood samples obtained during the study.
visits. Blood BHB levels will be determined through a coupled enzyme reaction using the BHB assay kit (Sigma-Aldrich®, USA)\textsuperscript{219}. In short, 0 to 10 μL of a BHB standard solution will be aliquoted into a 96-well plate and diluted to 50 μL using the BHB assay buffer. Blood serum will be deproteinized prior to addition to the reaction. Results will be determined by measuring absorbance at 450 nm.

\textit{Inflammatory biomarkers}: Inflammation, both systemic and neuroinflammation are strongly implicated in the pathophysiology of PD and increased inflammation is associated with faster disease progression\textsuperscript{46}. Dietary patterns such as the typical “Western diet” can induce pro-inflammatory events\textsuperscript{220}, while others such as the Mediterranean diets are anti-inflammatory in nature\textsuperscript{117}. In PD, higher baseline blood C-reactive protein (CRP) levels are associated with shorter survival\textsuperscript{118} and faster motor progression\textsuperscript{119}. We will perform the high sensitivity CRP (hsCRP) test to measure the levels of CRP. The blood samples will also be analyzed for 46 blood cytokine and chemokine analytes, using electrochemiluminescence assays from MesoScale Discovery, a high sensitivity assay kit.

\textit{3.2.5.3 Demographic and Clinical Information}

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

3.2.5.4 Statistical Analysis

We will employ repeated measures analysis of covariance where time (pre- versus post-) will be the repeated factor, and age, sex, PD duration, levodopa-equivalent dose, use of entacapone 69, and order of diet intervention will be covariates. Covariates that do not significantly contribute will be dropped from the model. We will perform separate repeated measures analyses for each diet intervention (pre- and post-MeDi-KD and MeDi-MCT, respectively) and will correct for multiple comparisons. The primary outcome analysis will be based on intention-to-treat. Datasets will also be analyzed by sex on an explorative basis. Functional analysis of altered microbiome profiles will be used to understand the biological mechanisms triggered by the respective dietary interventions. In-depth unsupervised analyses of clinical phenotypes, microbiome features, and inflammatory markers will be used to identify empirical groupings among features, interventions and patients.

3.2.6 Data Storage and Confidentiality

Each participant will be assigned a unique study ID upon entrance into the study. This number will not include any personally identifying information. Only this number will be used on any research-related information collected from participants, including bio-specimens. Only the PI and REB-approved study team members will have access to the key that links participant study numbers to their identifying information and the interim and final trial dataset. Clinical data collected from patient medical records will be de-identified upon extraction.
Data will be stored on Research Electronic Data Capture System (REDCap) as well as on password protected and encrypted computerized files on personal computers at the Djavad Mowafaghian Centre for Brain Health (DMCBH). Hard copies of participant source documents will be stored in a restricted-access office.

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

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

3.2.7 Adverse Event Reporting

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

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

If successful, this trial will de-risk future studies on ketogenic interventions by providing vital information about the safety, tolerability, adherence, and feasibility of the MeDi-KD and MeDi-MCT. We will determine if the ketogenic interventions in PD are safe or associated with harmful gut inflammation, reduction of butyrate/SCFA levels, systemic inflammation, gut barrier compromise or dysbiosis – particularly with respect to a potential *Akkermansia* bloom. Moreover, we will understand if the MeDi-KD and the MeDi-MCT can elevate blood ketone bodies to clinically relevant levels, while remaining feasible and safe.

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

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

3.4 Declarations

3.4.1 Ethics Approval and Consent to Participate

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

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

3.4.2 Funding

This study is funded by the Weston Family Foundation.
Chapter 4: Concluding Chapter

As Parkinson's disease (PD) becomes increasingly prevalent, the need for an effective neuroprotective treatment is becoming more urgent. While dopaminergic treatments provide symptomatic relief for motor-specific symptoms, they do not address non-motor symptoms, which have a remarkable impact on patients' quality of life. Additionally, since these therapies do not address the underlying causes of the disease their efficacy decreases over time as the disease progresses. The primary goal of this thesis was to investigate the potential of dietary interventions, specifically the MeDi and the KD, as supplementary therapeutic options for patients with PD.

The literature suggests that MeDi and KD can provide both motor and non-motor symptomatic relief for patients with PD. Furthermore, these interventions may slow disease progression by targeting some of the main biological mechanisms responsible for PD etiology.

For example, MeDi can help replenish the population of SCFA-producing bacteria in the gut microbiome of patients with PD via promoting the intake of fiber-rich foods. SCFAs, in turn supports the integrity of the gut barrier, prevents the infiltration of gut bacteria into the circulatory system, and reduces intestinal and systemic inflammation.

Similarly, KD can provide an alternative source of energy to the brain in form of KB, which can be used more efficiently than glucose. Incidentally, glucose metabolism is compromised in patients with PD due to impairments of mitochondrial complex I, an important component of cellular respiration. Additionally, ketone bodies can act as signaling molecules and contribute to the amelioration of disease-promoting mechanisms such as neuroinflammation and oxidative
stress. However, classic KDs may have undesirable effects on the gut microbiome, such as SCFA depletion, due to a lack of fiber-rich food intake. Combining the principles of MeDi and KD appears to mitigate these undesirable effects while retaining the benefits of a KD.

Thus, a new open-label, randomized-cross over clinical trial was designed to investigate the safety and feasibility of two Mediterranean-ketogenic interventions (MeDi-KD and MeDi MCT) in patients with PD. This trial will be the first to investigate Mediterranean-ketogenic interventions and MCT oil supplementation in patients with PD. The results of this trial will provide answers to many questions, including the effects of the interventions on fecal SCFA levels, gut inflammation, and gut barrier permeability, as well as the feasibility and desirability of adherence to these diets for patients with PD.

Dietary interventions offer a unique opportunity to address the biological underpinnings of disease in a relatively safe and risk-free manner. Unlike pharmacological treatments that target specific diseased systems, a healthy diet can have widespread beneficial effects on all bodily systems, making it a cardinal aspect of health, longevity and overall well-being. As Hippocrates, the father of modern medicine, wisely said, "Let food be thy medicine, and medicine be thy food."
References


89. Sarkar S, Malovic E, Harishchandra DS, et al. Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal


Appendix A: KIM Clinical Trial’s Consent Form

<table>
<thead>
<tr>
<th>Title of Study:</th>
<th>Ketogenic Diet Interventions in Parkinson’s Disease: Safeguarding the Gut Microbiome</th>
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| Place of Study: | Pacific Parkinson’s Research Centre  
Vancouver Coastal Health  
University of British Columbia (UBC) |
| Principal Investigator: | Dr. Silke Appel-Cresswell, MD, FRCPC  
Associate Professor  
Department of Medicine, UBC Hospital  
604-822-7754 |
| Co-Investigator(s): | Dr. Brett Finlay, PhD, OC, OBC, FRSC, FCAHS, Department of Biochemistry and Molecular Biology, Michael Smith Laboratories  
Dr. Wolfram Tetzlaff, MD, Dr. Med., PhD, Department of Zoology, iCORD  
Dr. Cheryl Wellington, PhD, Department of Pathology and Laboratory Medicine  
Dr. Tamara Cohen, PhD, Faculty of Land and Food Systems, BC Children’s Hospital Research Institute |
| Funding Source: | The Weston Family Foundation |

You are invited to participate in a research study conducted by the Pacific Parkinson’s Research Centre (PPRC) at the Vancouver Coastal Health Authority – University of British Columbia (UBC) site, Vancouver. It is important that you read and understand the following general principles that apply to all individuals participating in our studies:

a) Participation is entirely voluntary. You have the right to refuse participation.

b) You may withdraw from the study at any time without providing any reasons for your decision, and without jeopardizing your access to the medical care, education, or other service to which you are entitled or are presently receiving. The management of your medical care will not be affected in any way by your participation or non-participation in the study.

Invitation
You have been invited to participate in this study because you have Parkinson’s disease (PD).

You should be aware that there is a difference for both you and your doctor between being a patient and being a research participant. As a patient all medical procedures and treatments are carried out for your benefit only according to standard accepted practice. As a research participant you and your doctor also must take into account the requirements for the research study. These may include procedures and treatments that are not part of standard practice or are not yet proven.

The nature of the study, risks, inconveniences, and other important information about the study are discussed below. Please read this consent form carefully and feel free to ask any questions you may have to those discussing the project with you. If you wish to participate in this study, you will be asked to sign this form.

Please take the time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

**Who is conducting this study?**

This study is headed by Dr. Silke Appel-Cresswell and has received funding from the Weston Family Foundation.

**Background**

Parkinson’s disease (PD), the second most common and the most rapidly growing neurodegenerative disease worldwide. The underlying mechanisms of PD are poorly understood, and the majority of treatments are only targeted towards the symptoms. Gut-related symptoms such as constipation are common and often occur during the initial stages of the disease, suggesting a possible intestinal origin of PD. Over a dozen studies have demonstrated in the gut microbiome – the collection of microorganisms that live within the gastrointestinal tract – in PD.

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

Mediterranean diets are primarily but not exclusively plant-based with high intake of fiber content, which promotes many aspects of gut microbiome health. Adherence to Mediterranean diets is associated with a reduced risk of developing parkinsonian symptoms and higher age of PD onset.

Ketogenic diets are high in fat, adequate in protein and very low in carbohydrates. Unlike the Mediterranean diets, KD can provide ketone bodies as an alternative fuel source to glucose, the utilization of which is disturbed in the PD brain. Therefore, the KDs have the capacity to restore the brain energy deficits. Ketogenic interventions have been successfully used for decades in
epilepsy and more recently in diabetes, obesity, and neurological disorders. Pilot trials of KDs in PD report improved motor, non-motor, and cognitive symptoms.

Another method for increasing the ketone bodies is consumption of ketogenic medium-chain triglycerides (MCT). MCTs do not require limiting the carbohydrate intake and are therefore thought of as a more favorable option for increasing the ketone body levels. Although the effects of MCT supplementation are milder in nature, the ketogenic benefits seem to be largely preserved. The MCT oil supplements used in this trial will have Health Canada approval and will be used within their approved condition of use.

Several studies of classical KDs in non-PD populations have observed some alterations in the gut microbiome. The implications of these alterations are yet to be determined in the context of PD. By combining the ketogenic interventions with the Mediterranean diet, we hope to complement the ketogenic benefits of the former with the gut-health promoting effects of the latter. Our aim is to investigate these dietary interventions in 50 PD participants who will be enrolled in this trial.

**What is the purpose of the study?**

This is a Diet and Natural Health Product Trial. There is an established safe history of using both the interventions in human. The trial aims to investigate whether administration of the Mediterranean-Ketogenic diet and Mediterranean diet supplemented with medium-chain triglycerides is a safe treatment for those with Parkinson’s disease.

The aims of the study are:

1. To examine the safety of the ketogenic interventions with respect to gut health.
2. To examine the feasibility of adherence to the two diets.
3. To explore the clinical outcomes such as motor and non-motor (i.e., indigestion and constipation, quality of life, depression, fatigue, apathy, and cognition) PD symptoms, associated with administration of the two diets.

**Who can participate?**

You may be able to participate in this study if the following apply:

- Age between 40-85 years
- Diagnosis of Parkinson’s Disease based on Movement Disorder Society (MDS) criteria.
- Mild to moderate Parkinson’s disease (i.e., Hoehn and Yahr score of 1 to 3).
- On stable dopaminergic medication for at least one month

**Who should not participate?**

You will not be eligible to participate in this study if any of the following apply:

- You have atypical parkinsonism.
- Medical or psychiatric conditions that would prevent you from full participation in the dietary intervention.
- Pregnancy
- Significant difficulty with swallowing (i.e., dysphagia)
- Have diabetes and require insulin treatment.
- You have a known bleeding disorder or are on warfarin or other anticoagulant medications.
- You have inflammatory bowel disease (IBD)
- You have Dementia.
- You are unable to fill in electronic questionnaires or understand study instructions.
- Use of immunomodulatory agents
- Used probiotic in the last 4 weeks (yogurt, kefir, and other probiotic containing foods are allowed)
- Used antibiotic use in the last 3 months prior to the trial,
- Use of MCT oil or on ketogenic diet in the last 8 weeks prior to the trial.
- Are allergic to MCT oil, coconut oil, or coconut.
What does the study involve?

If you agree to be in the study, you will complete four study visits over a 24-week period that may include collection of data remotely (via electronic data capture, telephone, and videoconferencing using UBC Zoom) and in-person visits to the UBC Movement Disorder Clinic. Please note that the videoconferencing session will not be recorded.

You will be randomly assigned to either the Mediterranean-Ketogenic diet group (MeDi-KD) or the Mediterranean diet with MCT supplement group (MeDi-MCT). You will spend the first 8 weeks of the study in the assigned intervention group, before starting an 8-week washout period, where you will return to your typical lifestyle habits from before the study started. After the washout period you will spend an additional 8 weeks in the intervention group that you were NOT assigned to at the start. For example, if you started the study in the MeDi-KD group, you will now receive the MeDi-MCT intervention. This is called a cross-over design.

In the MeDi-KD group, you will adhere to a modified Mediterranean-ketogenic diet. The ketogenic component of the diet will require you to limit the intake of carbohydrates to about 10% of all calories consumed in a day, and obtain most of your energy from healthy fats, mostly from plant-based sources (~70-75% of your daily caloric intake) and lean proteins (~15-20% of your daily caloric intake). We will gradually increase the ratio of fat to carbohydrates during the first week from a 50-50 split (1:1 ratio) to 3-parts fat-1-part carbohydrates (3:1 ratio). The Mediterranean component of the diet will encourage you to consume more green, leafy vegetables, nuts, olive oil, and limit the consumption of processed or fried food, red meat, full-fat dairy, and sweets.

In the MeDi-MCT group, you will adhere to the Mediterranean diet as described above, but with a greater allowance for carbohydrates and natural sweets such as fresh fruits. In addition, you will be asked to take two daily doses of medium-chain triglyceride oil (MCT oil). MCT oil is extracted from coconut oil and will provide additional energy for your body. Additionally, MCT oil will work to mimic the ketogenic component of the MeDi-KD diet, albeit to a lesser extent. Please see below for more specific information on the MCT supplement.

During all the in-person visits (i.e., before and after the first and the second interventions) you will be asked to provide a blood and fecal sample and complete a test of memory, a motor and non-motor assessment of PD, and complete a series of questionnaires. The requirements for each visit will be explained in more detail later in this consent form.

All questionnaires and clinical assessments will be completed in the “ON” state, when you are feeling optimally medicated by your regular Parkinson’s medication(s).

Due to the nature of this study and its focus on safety, there will be no “blinding”; meaning that you and the investigators will always know which treatment group you are a part of.

Randomization
Randomization is making selections in a manner similar to flipping a coin. You will be paired to a random ID (by chance like the flip of a coin) and this way will be randomly assigned to either the MeDi-KD or the MeDi-MCT group to begin the study with. You will have a 50% of being assigned to the MeDi-KD and a 50% chance of being assigned to the MeDi-MCT first.

**Cross-over design**

In the first phase of the study, half of the participants will start the study in one intervention group and the other half will start the study in the other intervention group. After the washout, the groups will switch place and those who have receive intervention 1 will now receive intervention 2 and vice versa.

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<th>Post-intervention 1 (visit 2)</th>
<th>Pre-intervention 2 (visit 3)</th>
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<td>Movement Disorder Society - Unified Parkinson’s Disease Rating Scale (MDS-UPDRS)</td>
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<td>Questionnaires</td>
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<td>Fatigue Severity Scale (FSS)</td>
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<td>Parkinson Anxiety Scale (PAS)</td>
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<td>Starkstein Apathy Scale (AS)</td>
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<td>Beck Depression Inventory, 2nd Ed. (BDI-II)</td>
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<td>Parkinson’s Disease Questionnaire – 39 (PDQ-39)</td>
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<td>ROME III Constipation Module (ROME III)</td>
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<td>The Physical Activity Scale for Individuals with Physical Disabilities (PASIPD)</td>
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<td>Diet History Questionnaire II (DHQ-II)</td>
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<td>Bristol Stool Chart</td>
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<td>Compliance Survey</td>
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<td>Patient Global impression of Change (PGIC)</td>
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<td>WHO Quality of Life Questionnaire</td>
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Sample Collection

| Blood Sample | X | X | X | X |
| Fecal Sample | X | X | X | X |
| Qualitative Interview | | | x | x |

* These interventions will be done in person during visit one and will serve as both a screen tool and baseline measurements.

**Screening/Initial Study visit**

A member of the study team will review this study consent with you and will make sure to answer any questions that you have regarding participation in this study. The consent review discussion will take place remotely or in-person at the UBC Movement Disorder Clinic. Consent review will take 30 minutes to 1 hour, depending on the questions you may have about the study. Once you have agreed to participate in the study and signed this main study consent form (electronically or in-person), you will be asked to complete screening tools which include a cognitive assessment, the Beck Depression Inventory (BDI-II) and the Unified Parkinson’s Disease Rating Scale (UPDRS), as well as a past medical history and medication overview.

The consenting process, collection of health information, clinical interview and self-report questionnaires may be completed remotely via a secure electronic data capture system, telephone and/or videoconferencing, prior to a brief in-person visit at the clinic to complete procedures unable to be done remotely (i.e., blood sampling, provision of fecal sample kits and study product, etc.).

If you are a patient of the UBC Djavad Mowafaghian Centre for Brain Health (DMCBH), your clinical information related to your condition will be reviewed and stored for subsequent use in this study.

You must meet all eligibility criteria in order to participate in the study. If you do not meet eligibility criteria for this study, we will end study participation at this point. All procedures completed as part of the screening process are for research purposes only.

If you meet all the eligibility criteria for the study, you may continue the study visit. You do not need to answer questions that you are not comfortable answering. All testing will be done during “ON” periods, i.e., when the regular anti-Parkinsonian medication is working well. Please see the “Study Visit Summary” on page 6 for a more specific list of questionnaires, assessments, and other information we will be collecting.

If you agree to take part in this study, we will ask you to provide two biological samples per visit: a fecal sample and a blood sample. The blood samples will be collected during the study visit at the clinic and the fecal samples will be collected at home.
After you’ve met all eligibility criteria and completed all the initial visit procedures you will meet with a registered dietitian (RD) to discuss your dietary plans for either the MeDi-KD or MeDi-MCT interventions. The RD will provide you with meal plans and recipes and will be in contact with you throughout the study for additional support. You will also be provided with a study journal, which you will use to track your daily MCT intake (when in the MeDi-MCT arm), bowel movements, any adverse reactions, and record weekly one-day samples of your food intake. Moreover, you will be given a blood ketone self-monitoring kit, which you will use once weekly to determine your level of ketosis – a measure of how well the diet is working. This kit will include a blood ketone meter and test strips, in addition to a lancing device and lancets. You will use the lancing device to draw a small drop of blood from your finger, which you can then use to assess your diet’s success. Proper training and education on how to use the blood ketone self-monitoring device will be provided during the initial visit and upon your next pre-intervention study visit, should you wish to continue with your participation. For more information see the Blood Ketone Self-monitoring section below. Furthermore, you will receive two individual sessions with a registered dietitian within the first two weeks of each intervention, and weekly check-ins for the duration of each intervention thereafter.

If you start the study in the MeDi-MCT arm, you will also be provided with an 8-week supply of Nutiva MCT oil plus an additional supply for 7 days. You will be given specific instructions regarding the amount of MCT oil you should consume per day at different points of the study, depending on how your body reacts to the supplement. For instance, during the first week you will consume MCT oil in lower doses to help your body adjust to this supplement and to lessen the risk for gastrointestinal symptoms (see below for more information). Once/if your body acclimates to the supplement, you will start to increase the dose to up to 3 tablespoons per day.

You will receive a copy of this consent form along with the contact information of the study doctor (the principal investigator) and the study coordinator.

**Subsequent pre-/post-intervention visits**

**Visit 2/Postintervention Visit 1**

Following the completion of the first 8-week intervention, you will be asked to complete a second study visit (Visit 2/postintervention visit 1). Some of these visit procedures again may be completed remotely by accessing our electronic data capture system, by telephone or videoconferencing, prior to coming to the clinic for a brief in-person portion of the visit. The second stool sample will be collected at your first bowel movement following completion of the 8-week intervention and brought with you to the in-person visit (visit 2). We will ask you to complete a series of questionnaires, clinical assessments and provide a second blood sample for this visit. You will be asked to bring all study supplement boxes with all used and unused bottles to the in-person visit (if you started the study in the MeDi-MCT arm). At the end of the second visit overall compliance to the intervention will be assessed and you will be asked about
the feasibility and barriers to following the respective dietary intervention via a qualitative interview. A qualitative interview involves a research assistant asking structured open-ended questions regarding your subjective experience with the interventions and recording your response.

Washout period

After completing the second study visit, you will start the 8-week washout period. During the washout period you will return to the same lifestyle that you were following prior to the start of the study. We conduct phone check-ins with you at week 4 of the washout period to see how you are doing. There will be no in-person visits scheduled for the washout period.

Visit 3/pre-intervention visit 2

Following the washout period, you will be asked to complete another in person visit, which will be very similar in nature to the initial study visit. Namely, you will be asked to provide another blood sample, complete a number of questionnaires, and bring back the fecal sample that you have previously collected at home.

During this visit, you will be assigned to the other arm of the study to complete another 8-week intervention. If you have already completed the MeDi-MCT intervention, you will now meet with the RD again to discuss the meal plans and recipes for the MeDi-KD intervention. On the other hand, if you previously completed the MeDi-KD intervention, you will be provided with an 8-week supply of MCT oils in addition to the individualized session with the RD. You will be provided with another study journal, which you will use to track your daily intake of the MCT oil supplement, bowel movements, one day weekly records of your food intake, and any adverse reactions.

Final visit/post-intervention visit 2

After the second 8-week intervention is completed, you will be asked to complete a final visit that is similar to the previous postintervention visit (visit 2). Similar to before, you will complete a series of questionnaires and clinical assessments, provide a blood sample, and bring back the previously collected fecal sample. Another qualitative interview will be conducted to inquire about the feasibility and barriers of the second study intervention.

Both postintervention visits will be scheduled within the 8th week of the respective intervention period. Some of these visit procedures again may be completed remotely by accessing our electronic data capture system, by telephone or videoconferencing, prior to coming to the clinic for a brief in-person portion of the visit. The postintervention stool samples will be collected at your first bowel movement following completion of the 8-week intervention and brought with you to the visit.

Approximately 30 days after the final visit, the study coordinator will call you to see how you are doing.
Check-ins

On week 4 of each phase of the study (i.e., intervention 1, washout, and intervention 2) the study coordinator will call you to see how you are doing, ask about your compliance to taking the study treatment, and ask you to report any adverse events. You will also be asked to complete a series of questionnaires remotely from home (see study summary on page 6). The questionnaires are completed by accessing an encrypted link sent via email. This will allow you to complete the questionnaires on your computer directly into our research database.

How long does the study take?

The clinical research trial will take place over 24 weeks, with two 8-week interventions separated by an 8-week washout period. This study will include four visits that will include collection of data remotely prior to an in-person visit to the Movement Disorder Clinic. 8 weeks after each pre-intervention study visit, you will be asked to return to the Movement Disorder Clinic for your post-intervention visits. Again, data will be collected remotely prior to coming to the clinic. Each visit should take about 6 hours in total (includes both the in-person and remote data collection components) to complete. The questionnaires and phone call you will be asked to complete at the 4-, 12-, and 20-week time point will take about 1 hour to complete.

Additionally, you will have two one-on-one meetings with a registered dietitian during the first two weeks of the intervention followed by weekly check-ins thereafter (excluding the washout period). The one-on-one meetings and the weekly check-ins will take approximately 60 and 30 minutes respectively.

Medium-Chain Triglyceride oil supplement information

Name: Nutiva MCT Oil

Company: Nutiva Inc.

Dosage:

Week 1: will include pre-packaged MCT supplements in three gradually increasing doses. Starting with one teaspoon (5 mL) of MCT oil twice daily up to 1 tablespoon (15 mL) twice daily.

Week 2: Based on your blood ketone levels and tolerability of the supplement, you may or may not increase the dose to 1 tablespoon and 1 teaspoon of MCT oil (20 mL) twice daily.

Week 3 and beyond: either 15 or 20 mL of MCT oil depending on the previous week’s decision.

Ingredients: 100% fractionated coconut oil supplying 100% pure, true MCTs (i.e., only C8 and C10 medium-chain triglycerides)

Directions: to be taken twice daily in prepackaged doses. Can be added to shakes, coffee, or already-cooked meals. Not to be used for frying. First week doses should be taken with food.
**Mediterranean diet and Ketogenic diet information**

In the MeDi-KD group, you will adhere to a modified Mediterranean-ketogenic diet. The ketogenic component of the diet will require you to limit the intake of carbohydrates to about 10% of all calories consumed in a day, and obtain most of your energy from healthy fats, mostly from plant-based sources (~70-75% of your daily caloric intake) and lean proteins (~15-20% of your daily caloric intake). We will gradually increase the ratio of fat to carbohydrates during the first week from a 50-50 split (1:1 ratio) to 3-parts fat-1-part carbohydrates (3:1 ratio). The Mediterranean component of the diet will encourage you to consume more green, leafy vegetables, nuts, olive oil, and limit the consumption of processed or fried food, red meat, full-fat dairy, and sweets.

In the MeDi-MCT group, you will adhere to the Mediterranean diet as described above, but with a greater allowance for carbohydrates and natural sweets such as fresh fruits. In addition, you will be asked to take two daily doses of medium-chain triglyceride oil (MCT oil). MCT oil is extracted from coconut oil and will provide additional energy for your body. Please see above for more specific information on the MCT supplement.

**Blood Ketone self-monitoring**

You will receive a blood ketone self-monitoring kit upon your enrollment into the study. This kit will consist of a Freestyle Precision Neo device, Freestyle Precision blood ketone test strips, Freestyle lancing device, and Freestyle lancets. You will use this kit once per week to monitor your blood ketone levels, a measure of how well your diet is working. To do this, you will draw a small drop of blood from your fingertip using the lancing device and place on the test strip. The strip is then inserted into the reader, which will provide you with a measurement. We will provide you with all the necessary information to easily perform these tests. Once you finish your intervention, we ask you to bring the reader with yourself to the postintervention visit, where we extract your measurements from the device. No personal identifying information will be stored on the device and the measurements obtained will only be used for research purposes.

**Collection, analysis, and storage of specimens**

**Fecal sample kit:**

You will be provided with two fecal sample kits at each preintervention visit (i.e., visits 1 and 3) in order for you to collect the stool samples at home. The first stool sample will be completed within the first 2 days of your first study visit and mailed to UBC in a provided pre-packaged envelope. The second stool sample will be completed at your first bowel movement after the 8-week intervention is completed and brought with you to the final visit. The fecal sample kits will include a toilet accessory, spatula, sample tube, return packaging for the first sample, and instructions. For each sample you will be asked to take a scoop of your fecal matter and place
the scoop into the container. The fecal sample will only be used for analysis of bacteria, fungi, and immune markers.

Blood draw:
A total of 12 mL of blood, equivalent to approximately 1 tablespoon, will be collected by venipuncture at each visit. Blood collection will occur in the UBC Movement Disorder Clinic, located at DMCBH, by personnel certified in blood drawing and trained in biological safety. Proper sanitary and disinfection protocols will be followed stringently, and the procedure is considered to be minimally invasive and safe. Blood will be used for markers of inflammation, and molecules that may be produced or modified by the gut microbiome. The gut microbiome is the collection of bacteria and fungi that exist within the gut.

These samples are collected for research purposes and are not clinically validated. Therefore, we are not able to share the results of any testing (even for unanticipated findings) that is done on your samples.

The obtained samples will remain in the custody of the study principal investigator at all times and will be kept until data analysis of all your samples have been completed. Hereafter, the samples will be destroyed according to appropriate lab procedures for disposal of human biospecimen, unless you have separately consented to the future use of your samples.

All samples will be identified only by your unique study ID. We do not anticipate any linkage of the biospecimens with information regarding your identity. Fecal and blood samples will be stored at the DMCBH before analysis in restricted-access freezers. The blood and fecal samples will be transferred safely by a study team member from the DMCBH to the Michael Smith Laboratories or Microbiome Insights at UBC for analysis. All samples are stored in restricted-access freezers at their respective locations.

Samples will not be stored outside of Canada and will be destroyed in a confidential manner after the study has been closed. Dr. S. Appel-Cresswell will receive all reports about the research tests done on the samples. These reports will not be included in your health records.

The samples will only be used for the purposes described in this consent document and will not be sold or used commercially. Biobanking of your samples for unspecified, unrelated or genetic research will not occur. You have the right to withdraw your bio-specimen from the study at any time. Should you choose to withdraw before analysis, you will be asked if you would like your sample to be destroyed immediately. If you decide to withdraw your bio-specimen after it has already been analyzed, your bio-specimen will be destroyed if it has not already been, and the data from the analysis of your bio-specimen will be destroyed as well along with any clinical data already collected from you.

**What are possible harms and discomforts?**
MCT supplements are generally regarded as safe and are not associated with any significant side effects. Nutiva Inc.’s MCT Oil is a Health Canada Approved natural health product (NPN:80086912) and is commercially available across various Canadian stores. The most common associated side effect of MCT oil is gastrointestinal upset (i.e., diarrhea, bloating, vomiting, or nausea), which are typically mild and transient in nature and can be treated with over the counter (OTC) medication. These potential side effects are highly variable between individuals and may cause discomfort, at least during the initial phases of the trial until your digestive system becomes accustomed to the supplement. We will account for this by conducting regular check-ins and monitoring the potential symptoms you record in the study journal. Moreover, the dosage of the MCT supplement will be gradually increased during the first week of the study, which should allow your body to acclimate to the supplement.

MCT oil is high in saturated fat (14 g of saturated fats per 15 mL of oil) and its consumption in excess may increase the risk of developing coronary heart disease. Previous studies have determined that 50 g of MCT per day is safe and not associated with any blood lipid abnormalities. Moreover, you will receive continuous guidance from a registered dietitian who will ensure that your meal plans allow for the safe intake of the MCT supplement.

The Mediterranean ketogenic diet (MeDi-KD) is not associated with any significant risks. KDs in general rely on fats as the primary source of calories. High consumption of animal-based fats can be associated with an increased risk of developing coronary heart disease. Nonetheless, the majority of the fats in the Mediterranean-Ketogenic diet are derived from healthy plant-based sources to reduce the risk of hypercholesterolemia and other blood lipid abnormalities.

Classical KDs are associated with alterations withing the gut microbiome. Although it has not yet been established whether these alterations are harmful or not, the MeDi-KD circumvents these potential risks by allowing for a higher intake of vegetables, particularly those rich in fiber, which are essential for maintaining a healthy gut.

Nonetheless, we will monitor you closely through regular check-ins with the study team members and the registered dietitians. Particularly, we will actively monitor for gastrointestinal symptoms and “keto rash” – an inflammatory skin condition associated with classical KDs – which, responds to treatment with an increase in carbohydrate intake and antibiotics.

There might be slight discomfort associated with the weekly blood ketone self-monitoring tests, as you will be asked to use the lancing device to prick your fingertip and obtain a small drop of blood. Nonetheless, we anticipate that this potential discomfort will be minor in nature, as the Freestyle Lancing device II that we will provide you with utilizes a patented comfort zone technology, which has shown to reduce the pain associated with finger pricking.

We do not anticipate that the blood sample procedure will be associated with any serious complications. Infrequently, participants will experience pain, bleeding, swelling, infection, or bruising at the needle puncture site. Very rarely a participant will feel lightheaded and may
even faint when having blood drawn. Study team personnel will do what they can to make you feel comfortable.

In addition to the risks of physical harms outlined in this consent form, there are also possible non-physical risks associated with taking part in this study. Clinical questionnaires are also standard. If, during the administration of the questionnaires, you feel any discomfort, please do not hesitate to stop. You do not have to answer any questions you do not feel comfortable answering. There is no penalty for ending your participation in the study.

Notice of COVID-related risks: Given that this study requires in-person visits, there may be an increase in risk of exposure to COVID-19, including but not limited to risk associated with travel (e.g. using public transit) and increased exposure to other people in the research facility. We will be following our COVID-19 safety protocol throughout the study duration. Some of the COVID-19 safety measures that will be implemented include: reducing the in-person portion of each study visit by incorporating remote/virtual data collection session, pre-screening all participants for COVID-19 symptoms, and ensuring that all study team members who are involved in the in-person research are vaccinated against COVID-19 as required by the clinical research ethics board. If new information becomes available regarding a further increase in COVID-related risk, you will be informed and re-consented accordingly.

**Suicide Risk Management Protocol:** The study doctor has a suicide risk management plan in place in case significant suicidal thoughts or behaviors are disclosed, in which case the study doctor would be informed and would follow up with you to help ensure your safety and connect you to appropriate medical care.

**What are potential benefits of participating?**

You may or may not directly benefit from participating in this study. There is no guarantee that you will experience lessening of Parkinson’s disease-related symptoms following the start of the intervention. We hope that the information learned from this study can be used to develop a novel approach to the study and treatment of Parkinson’s disease that could benefit people with Parkinson’s disease in the future.

**What are the alternatives to the study treatment?**

Before you decide whether or not to be part of this study, your doctor will discuss the other options that are available to you. Although many dietary interventions exist, none are considered to be standard of care treatments for Parkinson’s disease-related symptoms.

**After the study is finished**

You may choose to continue adhering to the dietary regimens used in this trial, including supplementation with MCT oil, however, we strongly recommend you discuss any major changes in your lifestyle (including adding new supplements) with your primary health care
provider before implementation. The materials used in this trial are all available in Canadian
stores and can be purchased by anyone at their own discretion.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required
by U.S. Law. This website will not include information that can identify you. At most, the
website will include a summary of the results. You can search this website at any time.

What are my responsibilities?

You must be willing to attend the scheduled visits and will be asked to make every effort to
come to the research centre appointment on the day given to you. This is important for us to be
able to take the correct measurements at the correct time. It is also important that you take the
study treatment as directed. You will be asked to bring your supply of study treatment to the
research centre at the final visit (all used and unused bottles), so that we can know how much
you have taken. It is important that you tell the medical staff about any medications you are
taking before and during the study and follow any instructions you are given about them. You
should not take any other medications without first consulting the trial investigators. If
emergency medication is required the trial investigator needs to be informed immediately. This
may result in the participant’s exclusion or withdrawal from the clinical trial. Please discuss any
lifestyle or dietary changes (whether related to the trial or not) with the study doctor. Please
also tell the study doctor about any illnesses or side affects you have had since the last visit.

• You will be asked to provide truthful information about your medical history and current
  conditions.
• You will be asked to take the study treatment every day.

What happens if I decide to withdraw my consent to participate?

Your participation is completely voluntary. You may withdraw at any time without giving
reasons, without any change to the care you will receive from your doctor, and without any loss
of benefits to which you are otherwise entitled. You can do so by contacting the research
coordinator at xxx-xxx-xxxx or Dr. Silke Appel-Cresswell at xxx-xxx-xxxx. You may withdraw from
this study at any time without giving reasons. If you choose to enter the study and then decide
to withdraw at a later time, all information about you collected up to the point of your
withdrawal including, information obtained from your biological samples will be retained for
analysis in order to protect the integrity of the research, which may benefit future research
participants and patients. However, no further information will be collected.

If samples have been collected before you withdraw you will be asked if you would like them
destroyed. Your samples will only be kept with your permission if you withdraw. There may be
exceptions where the samples will not be able to be withdrawn for example where the sample
is no longer identifiable (meaning it cannot be linked in any way back to your identity).

Can I be asked to leave the study?
Under certain circumstances, the researchers might decide to end your participation in this research study earlier than planned. This might happen if you do not follow the study doctor’s directions, or if your medical condition changes so that staying in this study might risk your health or this research. The entire study could be discontinued at any time by the study doctor. If the study is stopped for any reason, you will be asked to have one final visit to ensure that your health has not been compromised. Assessments may be completed at this time point, which would include assessments and questionnaires outlined in the final study visit procedure section above. You must return all unused study treatment to the study investigator at your final visit, if applicable. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

**Will I receive new information about the study while participating?**

If we obtain any new information during this study that might affect your willingness to continue participating in the study, we’ll provide you with that information in a timely manner. When you are given this information, if you agree to continue in the study, you will be asked to sign an updated consent form. You may contact the study staff at any time after your participation ends to find out if any new information about this study has become available.

**How will my taking part in this study be kept confidential?**

Your confidentiality will be respected. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law. Your identity will not be revealed in such publications or other reports. The information will continue to be stored for a minimum of 5 years after any such publications or presentations.

Information collected from you will be de-identified as you will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an
opportunity to correct any errors in this information. Further details about these laws are available on request to the study team.

Your de-identified research data may be published or deposited into a publicly accessible location at the time of publication. Data could include questionnaire and clinical assessment scores. At no time will identifying information, such as your name, birth date or address be included in such data. This should not increase risks to you, but it does mean that other researchers may analyze the data for different reasons other than those described in this consent form. Once data is made publicly available, you will not be able to withdraw your data. The extent of the risk of you being identified through public data is unknown, but currently appears to be low.

This study does not involve any video recordings. Your audio will be recorded during the qualitative interview; however, this will be purely for transcription purposes. The audio recording will be destroyed immediately after it has been transcribed.

**Will I be reimbursed for my participation?**

There will be no charge to you for the research procedures. You will receive a pre-paid package for mailing your first fecal sample to the PPRC. You will not be reimbursed for the transportation expenses incurred, however, you will receive some remuneration, depending on where you will be travelling from (minimum of $30) to cover a portion of such expenses. This will be pro-rated across the study and provided to you upon arrival for each in-person visit. No receipts will be required for the remunerations. See below for details:

- Vancouver Metro Region = $30 per visit
- Fraser Valley Region= $45 per visit
- Vancouver Island/Kootenay-Boundary/Northern/Thompson-Okanagan Regions = $75 per visit

**Will the researchers have access to additional records?**

The researchers will have access to your medical records at the UBC Movement Disorder Clinic in order to obtain information on your medical history, clinical assessments routinely conducted in clinic and medications.

**Data storage:**

The information collected for this study visit will be stored on password protected computerized files in a de-identified manner on a secure server at the DMCBH at the University of British Columbia. Data may be additionally stored as hard copy files in locked cabinets in an access-restricted area at the DMCBH and on a secure database run by Vancouver Coastal Health Research Institute and housed on UBC IT servers. Your study information is stored only using a study ID that will not be linked back to any identifying information.

**What happens if something goes wrong?**
By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and that the following person can then be contacted for further information: Dr. Silke Appel-Cresswell at telephone number:xxx-xxx-xxxx. An emergency line that is available 24/7 is also listed on page one of this document.

**What will the study cost me?**

All research-related medical care and treatment and any related tests that you will receive during your participation in this study will be provided at no cost to you.

**Who do I contact if I have questions about the study during my participation?**

We encourage you to ask questions. If you have any questions or desire further information about this study before or during participation, or if you experience any adverse events, you can contact the research coordinator at xxx-xxx-xxxx.

**Who do I contact if I have any questions or concerns about my rights as a participant?**

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the UBC Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at xxx-xxx-xxxx (Toll Free: xxx-xxx-xxxx). Please reference the study number (H21-03747) when calling so the Complaint Line staff can better assist you.
Participant Information and Consent Form

Title of Study: Ketogenic Diet Interventions in Parkinson’s Disease: Safeguarding the Gut Microbiome (KIM Clinical Trial)

My signature on this consent form means:

- I have read and understood the information in this consent form
- I have had enough time to think about the information provided
- I have been able to ask questions and have had satisfactory responses to my questions
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes
- I understand that my participation in this study is voluntary and that I am free to withdraw from the research study at any time without explaining my decision to do so and without it impacting the quality of care that I receive
- I understand that I am not waiving any of my legal rights as a result of signing this consent form
- I understand that there is no guarantee that this study will provide any benefits to me.
- I have been told that I will receive a dated and signed copy of this form
- I voluntarily consent to participate in this study
- I authorize access to my health records and samples as described in this consent form
- I authorize overlapping assessments, information, and data with other studies that I have consented to at the Pacific Parkinson’s Research Centre to be pooled
- I authorize the use of videoconferencing for remote study procedures

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<th>Participant’s Signature</th>
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<th>Signature of Person Obtaining Consent (Principal Investigator or Designate)</th>
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Would you like to receive a copy of any publications related to this study after its completion?  
☐ Yes  ☐ No