Study Title: Brain Small Chain Fatty Acid Metabolism in Bipolar Disorder: Ketones

PI: Nicolaas Bohnen, MD, PhD

Study ID: HUM00227568

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Brain Small Chain Fatty Acid Metabolism in Bipolar Disorder: Ketones

This title should include, where possible, information on the participants, condition being evaluated, and intervention(s) studied.

Protocol Number: HUM00227568

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CONFIDENTIALITY STATEMENT

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Table of Contents

1 PROTOCOL SUMMARY

No text is to be entered in this section; rather it should be included under the relevant subheadings below. It may be useful to complete this section after the relevant sections in the protocol have been completed.

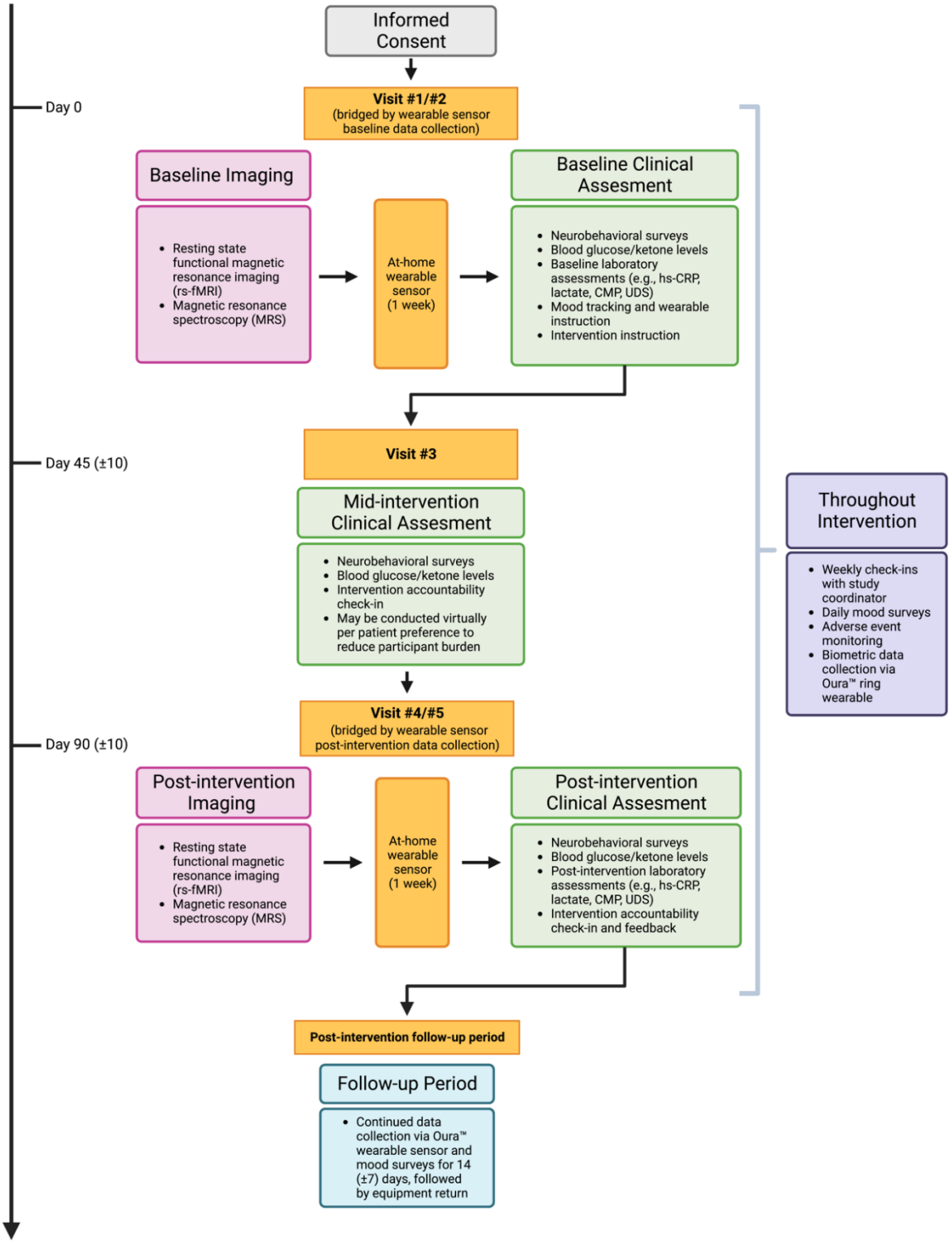
1.1 SYNOPSIS

Title:	Small Chain Fatty Acid Metabolism in Bipolar Disorder: Ketones
Study Description:	Biomechanistic pilot study study assessing ketone ester supplementation coupled with low glycemic index dietary changes in the setting of bipolar disorder
Objectives:	Primary Objective: Assess effects of ketone ester supplementation combined with low glycemic index dietary changes on blood ketones levels in persons with bipolar disorder. Secondary Objectives: Assess effects of ketone ester supplementation combined with low glycemic index dietary changes on blood glucose lability and fMRI-derived default mode network (DMN) neural network stability in persons with bipolar disorder. *Exploratory objective:* Explore mechanistic effects of ketone ester supplementation combined with low glycemic index dietary changes via various biomarkers, including MRS-derived brain ketone body uptake, inflammatory markers, and peripheral blood markers of mitochondrial function such as lactate.
Endpoints:	Primary Endpoint: Statistically significant increase in average blood beta-hydroxybutyrate level compared to baseline Secondary Endpoints: Statistically significant decrease in average blood glucose lability compared to baseline; statistically significant increase in computed fMRI DMN neural network stability compared to baseline
Study Population:	12 individuals with bipolar disorder (any subtype)
Phase or Stage:	Phase 1b
Description of Sites/Facilities Enrolling Participants:	University of Michigan Functional Neuroimaging, Cognitive, and Mobility Laboratory & Heinz C. Prechter Program for Bipolar Research
Description of Study Intervention/Experimental Manipulation:	Ketone ester supplementation (19 g BID PO [12.5 g active ingredient per serving] of Qitone 'Pro-Ketone' powder, a sports and cognitive performance supplement widely available over the counter that has been the subject of numerous published studies OR, in the case of Qitone availability shortage, 20 g BID PO [10 g active ingredient per serving] of KetoneAid Ke4 Pro Ketones) in combination with four dietary changes (not consuming candy/sweets, not consuming soda, replacing 'white' grains with complex carbohydrate sources such as brown rice or quinoa, if eating fruit consuming it at the end of the meal) designed to reduce glycemic spiking for a duration of 90 days
Study Duration:	2-3 years

Participant Duration: 3.5-4 months

1.2 SCHEMA

Figure 1. Protocol Flow Diagram



* Baseline imaging includes optional 18F-BCPP-EF PET scan to assess mitochondrial complex I function at baseline.

1.3 SCHEDULE OF ACTIVITIES INCLUDING EXPLORATORY OUTCOME PARAMETERS

	Eligibility Visit *	**Visit 1* Baseline Imaging**	**At-home wearable sensor use (7 days ± 3 days) before starting KE intervention**	**Visit 2* Baseline Clinical Assessment**	**At-home KE intervention 90 days ± 10 days (to continue throughout rest of study)[3]**	**Visit 3 Mid-intervention Clinical Assessment @ day 45 ± 10 days**	**At-home wearable sensor use (7 days ± 3 days) while on KE intervention**	**Visit 4* Post-intervention Imaging (while on KE intervention)**	**Visit 5* Post-intervention Clinical Assessment (while on KE intervention)**	**Post-intervention devices**
Overview	Eligibility screening	Baseline Imaging: fMRI and PET; instructions for wearables	Continuous glucose monitoring (CGM); sleep tracking ring (Oura)	Baseline assessment; psychological and mood surveys; bloodwork; CGM, Oura, & glucose/ketone meter instructions	KE supplement taken twice daily with meals (e.g. with breakfast and dinner)	Repeat psychological testing (may be completed virtually if needed); repeat glucose/ketone meter measurements	Continuous glucose monitoring (CGM); sleep tracking ring (Oura)	Post-Intervention Imaging: fMRI	Post-Intervention Clinical Assessment	Return Oura ring (via mail or drop off per participant preference)
Approximate time commitment	30-60 minutes	3-6 hours	-	4-6 hours	-	2-4 hours	-	2-4 hours	4-6 hours	15 minutes
Test location	Domino's Farms or Virtual	UM Hospital	Home	Domino's Farms	Home	Domino's Farms	Home	UM Hospital	Domino's Farms	Home
Informed consent (or prior eConsent)	X	X								
fMRI scan		X						X		
OPTIONAL [18F]BCPP Baseline PET scan		X								
Continuous glucose monitoring		X	X	X			X	X	X	
Sleep Tracking Ring (Oura)			X	X	X	X	X	X	X	X
Glucose/ketone meter[2]				X		X			X	
Blood draw[1]				X					X	
Urine drug screen	X*	X*		X*					X	

Neurobehavioral surveys	X			X		X			X	
Optional diagnostic confirmation interview (MINI, DIGS or SCID-V)	X									
Mood tracking (via app on phone)			X	X	X	X	X		X	
Meal Photo-Diary (via app on phone)					X	X	X	X	X	
In lab/at home KE intervention usage				X	X	X	X	X	X	
At home diet modification[3]					X	X	X	X	X	
Weekly phone / email monitoring					X					
Adverse event assessment**		X		X		X		X	X	
Drug accountability				X					X	
Medication review	X	X		X		X		X	X	
Pregnancy test in women of childbearing potential		X (within 48 hours of optional PET)								

* Visits 1-2 and 4-5: There is no pre-set sequence for which assessment (clinical or imaging) comes first. Prior to imaging visit, an eligibility visit will take place (which can be done virtually) to perform a suicidality screening and substance use history screening if applicable. An optional diagnostic confirmation interview (MINI, DIGS or SCID-V) administered by a trained clinician may also take place at the eligibility visit, but may be skipped to reduce participant burden if pre-existing diagnostic confirmation is available (e.g., if the participant already received diagnostic confirmation via the Longitudinal Study of Bipolar Disorder [HUM00000606]). Informed consent may be administered at this time or, alternatively, directly prior to initiating the first visit patient preference. Any questions will be answered during this time and participants will have an opportunity to get acquainted with the protocol prior to beginning the study. While UDS may be included in this eligibility assessment, it may alternatively be performed at either Visit 1 or 2 to accommodate participant schedules/logistical hurdles.

**In addition to adverse event assessment taking place during visits, adverse event monitoring will also take place during weekly phone check-ins with study coordinators. The SAFTEE form will be used to screen for adverse events.

[1] Blood laboratory assays will be optional for participants (e.g., to accommodate participants with a strong preference to avoid blood draws or a specific phobia pertaining to blood). Participants may be required to fast in the morning prior to blood draw and/or abstain from exercise for at least several hours prior to blood draw.

[2] Glucose/ketone meter measurements will be performed at baseline, during the intervention (approximately mid-way at Day 45 ± 10 days), and at the end of the intervention period. Measurements will be taken before supplementation (morning fasting) and two hours post-supplementation. Given the speed and

ease of obtaining glucose and ketone levels with the Keto-Mojo device, participants will be provided the option to obtain more frequent glucose/ketone measurements (e.g., once weekly or daily) if desired, though they will not be required to obtain these additional measurements.

[3] Modified diet throughout KE intervention to minimize blood sugar spiking. Participants will be asked to follow a modified low-glycemic index diet in which they cut out soda/sugary drinks, candy, and white grains/rice. If consuming fruit, they will be asked to do so at the end of each meal to minimize glycemic spiking. If they desire more information, participants may be given a recipe sheet with off-limit foods/drinks and alternative meal/snack ideas.

2 INTRODUCTION

2.1 STUDY RATIONALE

Bipolar disorder is a disabling chronic condition that poses unique challenges to treatment [***1***,***2***,***3***,***4***]. Treatment of bipolar depression is less well investigated than treatment of unipolar depression, with antidepressant treatment bearing additional risks of evoking mania, destabilizing mood, and inducing dysphoria or potential suicidality in the context of bipolar disorder [***5***,***6***,***7***,***8***]. On the other side of the spectrum, it is not uncommon for patients to exemplify inconsistent (or nonexistent) adherence to a mood stabilizer regimen for the treatment of mood elevation. Among patients with bipolar disorder, doubts about the need for medications as well as concerns for adverse effects have been cited as drivers of markedly high rates of medication nonadherence, which may approach 50% (or potentially as high as 69% by some estimates) [***9***,***10***].

With a patient-centered approach to care, global functioning represents another important consideration alongside symptomatic relief. In a study that followed patients for 2–4 years after their first hospitalization for mania, 28% of patients remained symptomatic and 57% did not return to their prior level of functioning [***11***]. While pharmacological breakthroughs have provided undeniable value and saved countless lives, these findings suggest the possibility that existing paradigms may be further improved upon as our understanding of this disorder evolves.

What remains unknown is how exactly clinical practice can be further advanced, particularly in light of an evolving pathophysiological understanding of bipolar disorder. While emerging evidence supports a potential role for metabolic interventions such as a ketogenic diet in the treatment of this complex disorder, this possibility raises further questions related to policy challenges and insurance reimbursement hurdles affecting the delivery of care for nutrition-related interventions. Whereas nutrition-related interventions offer the possibility of transformative pleiotropic benefits, they are also considered to be more complex to incorporate into research methodology and clinical practice as compared to pharmaceutical treatments. Adherence represents a key consideration in psychiatric populations, providing an impetus to consider interventions that may be more easily implemented for patients who struggle with self-discipline or are in the midst of significant life stressors. Drawing on this line of reasoning, investigation of exogenous ketone supplements may reveal strategies for improving the accessibility of ketosis. To the authors' best knowledge, there is not a single clinical study investigating exogenous ketone supplementation in the setting of bipolar disorder to date.

Importantly, the effects of exogenous ketone supplementation are widely known to be modulated by dietary intake (in particular, glycemic/insulin spiking). Exogenous ketone supplementation without any other dietary changes may thus confer limited benefit depending on the clinical context. As a potential middle ground, combining exogenous ketone supplementation with low glycemic index dietary changes may mimic two foundational components of strict ketogenic diets: namely, high levels of ketones and minimal glycemic/insulin spiking. This pilot study thus aims to investigate the biomechanistic effects of exogenous ketone supplementation combined with low glycemic index dietary changes in the setting of bipolar disorder. Specifically, we will investigate the effects of this intervention on neural network stability, systemic inflammation as measured via high-sensitivity C-reactive protein, and mitochondrial function (assessed peripherally via lactate metabolism) as exploratory outcomes. Tolerability and feasibility of this intervention in the setting of bipolar disorder will also be explored.

2.2 BACKGROUND

Although the exact mechanisms underlying bipolar disorder remain unclear, it is evident that agents implicated in neuronal membrane stability are effective in mitigating symptoms [***12***,***13***,***14***,***15***,***16***]. The overlapping pharmacotherapies utilized for both bipolar disorder and seizures suggest that a mechanistic overlap may exist between these conditions. A corollary hypothesis derived from this possibility is that treatments with proven efficacy in the realm of seizure management may also prove to be beneficial for patients with bipolar disorder. Interestingly, one of the most time-proven approaches to seizure control is the induction of ketosis, with Hippocrates himself documenting fasting as a remedy for seizures [***17***]. In 1921, Dr. Russell Wilder at the Mayo Clinic postulated that the benefits of fasting may be dependent on high levels of ketones in the blood, speculating that similar benefits may be derived if ketonemia was achieved by other means [***18***]. Hence, the ketogenic diet was born, with breakthroughs that followed in the treatment of seizures.

With these considerations in mind, the ketogenic diet may also enact biomechanistic effects in the setting of bipolar disorder. This hypothesis has been supported by limited preliminary data. In a study by Phelps et al. [***19***] two women with bipolar disorder were able to maintain the ketogenic diet for at least two years. Both women tolerated the diet well and achieved mood stabilization exceeding that attained with medication, reporting subjective improvement distinctly related to ketosis. Ultimately, both patients were able to discontinue mood-stabilizing medications and maintain mood stability with the ketogenic diet as a stand-alone intervention. In a data mining analysis comparing hundreds of anecdotal reports from online bipolar disorder forums, Campbell et al. [***20***] found that reports of symptomatic remission were significantly higher for the ketogenic diet in comparison to other health-conscious diets (with approximately 73% of reports endorsing some degree of mood stabilization with the ketogenic diet, as compared to approximately 54% of reports endorsing some degree of mood stabilization with other health-conscious diets). More recently, a retrospective analysis that included 13 patients with bipolar disorder reported significant clinical improvements when the ketogenic diet was utilized in an inpatient setting (depressive symptom severity was reduced by over 60%, with concordant improvements in clinical global impression) [***21***].

Conceptually, there are other reasons to investigate the effects of ketogenic interventions in the setting of bipolar disorder. Accumulating evidence has provided increasing support for the hypothesis that mitochondrial dysfunction may be an underlying feature of bipolar disorder [***22***,***23***,***24***]. Notably, mitochondrial dysfunction has also been implicated in the pathogenesis of epilepsy [***25***]. Considering the profound mitochondrial effects of ketogenic interventions, it is plausible that improved bioenergetic efficiency attained via ketosis may promote stabilization of cellular functioning and, thereby, clinical improvement. Notably, the Na^+/K^+ ATPase, responsible for regulating neuronal membrane potential, represents a major beneficiary of bioenergetic availability. By some estimates, this ATPase consumes nearly half of the ATP in the brain [***26***]. In a setting of Na^+/K^+ ATPase hypofunctionality, sodium would be expected to accumulate within neurons, altering resting potential (and, thereby, altering patterns of neuronal excitability). Interestingly, individuals with bipolar disorder have been observed to exhibit decreased Na^+/K^+ ATPase activity and increased intracellular sodium concentrations [***27***]. Moreover, mood stabilizers shown to be effective in bipolar disorder converge upon a shared mechanism: reduction of intracellular sodium concentrations [***14***,***28***,***29***,***30***]. Similarly, ketogenic interventions may also converge upon this mechanism. In addition to powering the Na^+/K^+ ATPase by supporting mitochondrial energetic output, ketosis may also lower intracellular sodium by inducing a mildly acidotic state, as extracellular protons will be exchanged for intracellular sodium [***31***,***32***,***33***]. An acidic neuronal environment also reduces neuronal excitability via a variety of other mechanisms, including modulation of calcium channel conductance [***34***]. Furthermore, a ketogenic diet may elevate GABA/glutamate

ratios, an effect mediated by the gut-brain axis [***35***,***36***,***37***]. Modulation of neuronal excitability is thus a plausible mechanism attributable to ketogenic interventions, particularly in light of the clinical outcomes achieved by the ketogenic diet in the realm of epilepsy.

Altered patterns of synaptic plasticity have also been identified in the setting of bipolar disorder, bearing implications for interventions that modulate synaptic plasticity [***38***]. Animal models have demonstrated that ketone bodies are capable of stimulating brain-derived neurotrophic factor (BDNF) production [***39***,***40***], converging with evidence in human research [***41***,***42***,***43***]. BDNF is widely known as a vital modulator of synaptic plasticity.

Looking beyond the level of the synapse, a growing body of functional neuroimaging research has revealed that bipolar disorder is characterized by abnormalities in neural network functional connectivity. In particular, altered interactions between the amygdala and prefrontal regions have been implicated in bipolar disorder [***44***]. Via an elegant fMRI analysis, Mujica-Parody et al. [***45***] found that the ketogenic diet has a stabilizing effect on neural network functional connectivity. Moreover, exogenous ketone esters also demonstrated a stabilizing effect on neural network functional connectivity, even if consumed in the setting of a regular diet. Theoretically, the stabilization of neural networks may also bear implications for the management of bipolar disorder. This line of reasoning would be consistent with patterns of altered neuronal excitability identified in the setting of bipolar disorder [***46***], as altered patterns of spreading activation would be an expected downstream effect of altered neuronal excitability.

Furthermore, with a growing body of evidence demonstrating intersections between neuroinflammatory processes and psychiatric disorders, it is plausible that the anti-inflammatory properties commonly attributed to ketone bodies may also reduce inflammation in the setting of bipolar disorder [***47***,***48***,***49***]. Neuroinflammatory processes have been implicated in the pathogenesis of the bipolar disorder, as demonstrated via markers of excitotoxicity and neuroinflammation mediated by the IL-R cascade [***50***]. Ketone bodies have been shown to inhibit activation of the NF-κB inflammasome (a key orchestrator of this signaling cascade) [***51***,***52***].

Intertwined with all of these factors, a final consideration is the role of epigenetics in the pathophysiology of bipolar disorder. In light of the clear impact of environmental factors on this episodic disorder, it is plausible that fluctuations in epigenetic expression may underlie shifts in symptomatology. Of particular relevance to this trial is the finding that ketone metabolism drives activation of sirtuins (which have been referred to as “the guardians of the genome” given their central role in epigenetic regulation), in addition to modulating epigenetic expression via a variety of other mechanisms [***53***,***54***,***55***,***56***].

While accumulating evidence thus supports a role for metabolic interventions such as a ketogenic diet in bipolar disorder continues to accumulate, these findings highlight the remaining gaps in our knowledge and unanswered questions. Importantly, there is not a single study assessing ketone ester supplementation in the setting of bipolar disorder to date. Mechanistically, the evidence to date suggests that some reduction in glucose spiking may be necessary for benefits to be derived from ketone ester supplementation (i.e., taking the supplement without making dietary changes may not offer much benefit), but this needs to be formally investigated. This pilot study will thus serve as a first step in addressing some of these unanswered questions.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

General risks:

- There is a rare risk that participants may experience some minor anxiety ('test anxiety'), become worried, or have an anxiety reaction in response to any of these tests and procedures. For example, participants may become worried about their health, or you experience a sudden fear of the confined space while in the scanner.
- *Trained research staff will conduct all tests and procedures. The staff will be prepared to respond to anxiety, concerns, and behavioral changes by temporarily suspending testing, breaking up testing sessions into several brief visits if needed, and/or answering questions. During the PET and MRI scans* participants *will be able to talk to technologists and indicate right away if they wish to stop the study and leave the scanner. At the option of a personal physician, they may be prescribed sedation with lorazepam (Ativan) or diazepam (Valium) to be taken before the scan in accordance with the prescription directions.*

- During the course of the study, participants may receive several blood draws, intravenous (IV) catheters, and injection for the PET radiotracer. There is an infrequent risk of bruising, bleeding, infection, or soreness at the injection site. There is a very rare risk for infection. There is a rare risk that participants may feel dizzy, lightheaded, or faint after an injection.
- *Blood draws, IV catheter insertions, and injections will be performed by a certified and experienced research technician or other health care professional who is also trained in bloodborne pathogens control. Aseptic techniques will be used in accordance with University of Michigan guidelines.* Participants *can lie down if they feel dizzy, lightheaded or faint after an injection. Moreover, blood draws and the PET imaging portion of the study are optional for participants, and it is possible to participate in the study without completing the PET scan or blood draws (for example, to accommodate a specific phobia of blood or patient preference to avoid any degree of radiation).*

- None of the test results, brain images, and procedures in this study will be reviewed or interpreted for making a medical diagnosis. For example, there is the potential that the MRI scan may reveal an abnormality that is already in a participant's body, such as a cyst or tumor. Any result or abnormality that would be indicative of current or future disease will most likely not be discovered. Many such abnormalities are not clinically significant, but participants may need or want to investigate them further. Such a finding might require additional studies, and maybe even treatment, which would not be paid for by the investigators, the sponsor, or the University of Michigan. The research results of the brain images and genetic testing will NOT be communicated back to participants.
- *Participants should consult their personal doctor if you have any health concerns.*

Clinical tests:

- There is an infrequent risk of physical fatigue during the clinical examination.
- *Trained research staff will conduct all the tests and administer all the questionnaires. The staff will be prepared to respond to participant concerns by temporarily suspending testing and/or breaking up testing sessions into several brief visits if needed.*

Neurobehavioral tests:

- There is an infrequent risk of boredom, frustration, and/or mental and physical fatigue during the neurobehavioral testing.

- *Trained research staff will conduct all the tests and administer all the questionnaires. The staff will be prepared to respond to participant concerns by temporarily suspending testing and/or breaking up testing sessions into several brief visits if needed. If needed, questionnaires may be completed over the phone/Zoom video call.*

Ketone ester drink side effects:

- Ketone ester supplements are usually well tolerated. However, possible infrequent side effects may include mild nausea, diarrhea, indigestion, upset stomach, distended abdomen, feeling bloated, heartburn, loose bowel movements, constipation, passing gas, keto flu-like symptoms, headache, dizziness, headrush (feeling of warmth or buzz in the head lasting a few minutes), or sleep changes. Ketones may affect blood glucose levels (decrease or increase), which could lead to symptoms of lightheadedness or jitteriness. Participants may also feel more mental or physical energy with supplementation. Theoretically, increased energy availability provided by ketones may be a risk factor for hypomania (which, in turn, is a risk factor for social consequences and impulsive behaviors such as impulsive spending, risk-taking, and sexual promiscuity), though we are not aware of any clinical reports that validate the possibility of hypomania induced by consumption of exogenous ketone supplements to date. The ketone ester may have a poor or bitter taste, but the drink can be diluted.
- *The ketone ester dose in this study will be a standard dose that does not exceed the amount that has been deemed safe for consumption. The manufacturer of this supplement has published numerous studies related to safety and tolerability, which was essential to our selection process when choosing a supplement for this study. Nonetheless, weekly phone check-ins will be provided by study coordinators to monitor for any side effects, in addition to adverse effect monitoring taking place during every visit. If participants experience any of the above symptoms, they will be instructed to contact our team immediately for next steps. Participants may be advised to stop taking the supplement (temporarily or permanently) or to seek medical care if necessary.*

MRI scan:

- There is an infrequent risk of discomfort or anxiety from being in the confined space of the MRI scanner.
- *We will provide pads and blankets to make you as comfortable as possible. Participants will be able to talk to a technician throughout the study, and will be able let the technician know right away if they want to stop the study and get out of the scanner. At their request, they may be provided with a mild sedative; however, in that case prior arrangements to be driven home by an accompanying adult must be made.*

- The MRI scanner makes loud, vibrating noises.
- *Participants will wear foam earplugs to reduce the loud noises made by the scanner and prevent any hearing damage.*

- Some studies, like this one, have the potential to cause "peripheral nerve stimulation" (PNS). PNS is a light touching sensation on the skin surface, lasting only for a few seconds. It may cause mild discomfort, but is not harmful.
- *The MRI machine is operated within FDA guidelines so the potential for inducing PNS is low.*

- Sometimes, subjects report a temporary, slight dizziness, light-headedness, or nausea during or immediately after the scanning session.
- *If* participants *feel dizzy or light-headed, they will have you get up slowly from the scanner.*

- Because the strong electromagnetic fields can move metal objects and cause heating, there is a risk that loose objects (jewelry, keys) outside the body could be accelerated by the magnetic field and strike participants, causing injury. There is also a risk that the magnetic fields could disturb a metal fragment in the body, interfere with an implanted device, such as a pacemaker or neurostimulator, or cause metal (including foil-backed medication patches) on or in the body to heat up, causing harm.
- *We keep the environment around the MRI scanner completely free of loose metal objects that could be moved by the magnetic field, and we will make sure that* participants *have no metal on their bodies that could be affected by the MRI scanner. We will also ask participants questions and have you complete an MRI screening form to make sure that they have no metal inside their body that would cause harm during the MRI scan. The radiologist may order an X-ray to make sure there are no metallic fragments in a given participant's eyes or chest.*

PET scans:

- There is an infrequent risk of bruising, bleeding, infection, or soreness associated with intravenous catheter placement, similar to the risks associated with routine blood testing. Also, participants may feel dizzy or lightheaded or may rarely even faint when the tube is put in or taken out.
- *We will use highly trained personnel for placement and removal of the IV.*

- There is a very rare risk that participants could experience an allergic reaction to the PET tracer. This could involve itching, skin rash or shortness of breath shortly after injection. However, because of the very small tracer amounts used in PET imaging, the risk is very rare.
- *Certified staff will be in attendance at all times during the study. A physician will be available, and an emergency cart is located in the PET Facility for treatment of any adverse reactions that may occur.*

- There is an infrequent risk of discomfort or anxiety from being in the confined space of the PET scanner.
- *We will provide pads and blankets to make participants as comfortable as possible. They will be able to talk to a technician throughout the study, and you will be able let him/her know right away if they want to stop the study and get out of the scanner. At their request, they may be provided with a mild sedative; however, prior arrangements to be driven home by an accompanying adult must be made in that case.*

If participants opt to complete the optional PET scan in this study, they will be exposed to radiation from the CT scan (embedded in the PET scanner) and the [18F]BCPP-EF radiotracer. The risks associated with the amount of radiation exposure participants receive in this study are considered very rare and comparable to everyday radiation exposure risks. Participants will inform the investigators if they have had any major radiation exposure in the past, particularly in the past year, such as medical treatment with X-rays or radioactivity, or diagnostic X-rays, CT-scans, or nuclear medicine scans.
The risk of biological effect from radiation exposure in humans is measured in terms of Sieverts (Sv) or mSv (1/1000 Sv), which is a unit of uniform whole-body exposure. The radiation exposure received from

the CT and [18F]BCPP-EF PET scan is equivalent to a uniform whole-body dose of 2.48 mSv, which is approximately 4.9 % of the annual radiation exposure (50 mSv) permitted to radiation workers by federal regulations. This amount is approximately the annual exposure received from natural background radiation levels. Participants will be instructed to use the bathroom and urinate as soon as possible after the PET scan in order to minimize bladder exposure.

The exposure resulting from this PET scan in a single year is about 1 time higher than the natural background exposure.

In the case of technical failure of the scan participants may be asked to undergo a repeat [18F]BCPP-EF PET scan. This may expose these participants who repeat the scan to an additional 2.48 mSv of radiation from internal and external exposures.

There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (cellular abnormalities) or cancer. However, the risk associated with the amount of radiation exposure that participants will receive from this study is considered to be low. The risk of a side effect from this level of radiation exposure is very rare. The risk from radiation exposure of this amount is considered to be similar to other everyday risks, such as driving a car.

- No PET studies will be performed on pregnant, nursing, or potentially pregnant women.

A urine pregnancy test will be performed on all women of childbearing potential within 48 hours prior to the PET scanning session. If the test is positive for pregnancy, they will not be able to complete the study and no radioactive drugs will be injected. PET imaging will also not be performed in breastfeeding women. As mentioned above, PET imaging is optional and participation in the study is still possible without completing the PET Scan.

Genetic testing:

- We may test for mitochondrial genes that are related to metabolism. There is a very rare risk that the genetic information we obtain from samples could prove embarrassing to participants, if somebody were able to link the genetic information with them.
- *We have a system of double-coding the genetic information, so that it is extremely unlikely that the genetic information would be connected with participants. Most importantly, we will break the link between the genetic information and participants once the study is completed.*

Continuous glucose monitoring: There is a small risk of pain when applying the sensor. Participants may occasionally feel a tinge of pain or discomfort when wearing the monitor. There is an infrequent risk of an allergic reaction or skin irritation to the covering tape. Signs of skin irritation will disappear once participants remove the sensor.

Wearing the Oura ring sleep/physical activity tracker will not cause any risks beyond those associated with wearing a normal ring. Like with every ring one may wear on a finger, there is a small risk that that it may feel tight or that participants may have difficulties removing it. Participants will be individually fitted for the ring to minimize this risk.

As with any research study, there may be additional risks that are unknown or unexpected.

The researchers have taken steps to minimize the risks of this study. Even so, participants may still have problems or side effects, even when the researchers are careful to avoid them. Participants will be

instructed to tell the researchers about any injuries, side effects, or other problems that they have during this study. They will also be instructed to tell their regular doctors.

Being in more than one research study at the same time, or even at different times, may increase the risks to participants. It may also affect the results of the studies. Participants should not take part in more than one study without approval from the researchers involved in each study.

2.3.2 KNOWN POTENTIAL BENEFITS

Potential benefit of improved sleep, cognition, mood stability, and gastrointestinal health from taking ketone ester. Potential benefit of improved glycemic control and metabolic/cardiovascular/general health from reducing glycemic spiking due to dietary changes.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Collectively, this study involves a minimal increase above baseline risk for participants. The ketone ester supplement used in this study is widely available over-the-counter and has been safety tested by the manufacturer in cGMP-compliant facilities. Minimizing soda and candy intake poses minimal risk to participants (if anything, this may provide health benefits). The intervention proposed for this study thus has a notably favorable safety profile compared to most investigative pharmaceutical trials. While the PET scan in this study involves a degree of radiation, the dose for this particular tracer is relatively low and completing this scan is optional for participants; participants may thus choose to participate in the study without completing the scan if they wish to avoid any degree of radiation. A summary of ways that risk to participants were minimized in the study design has been provided above in patient-friendly language for each corresponding risk. The biomechanistic insights derived from this study design may help advance treatment paradigms for patients suffering from bipolar disorder, justifying the minimal increase above baseline risk.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To assess biomechanistic effects of ketogenic-mimicking diet on blood ketone level	*Blood beta-hydroxybutyrate level after supplementation period compared to baseline*	*The ketogenic diet includes two foundational components: high blood ketones and low blood glucose levels. Our aim is to assess whether we can mechanistically approximate these conditions with an approach that combines supplementation of exogenous ketones with dietary changes intended to reduce glycemic spiking.*
Secondary		
To assess biomechanistic effects of ketogenic-mimicking diet on blood glucose level	*Blood glucose stability (as ascertained via CGM) after dietary*	*The ketogenic diet includes two foundational components: high blood ketones and low blood glucose levels. Our aim is to assess whether we can*

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	change period compared to baseline fMRI-derived computed DMN stability	*mechanistically approximate these conditions with an approach that combines supplementation of exogenous ketones with dietary changes intended to reduce glycemic spiking.* *We will also assess mechanistic effects on DMN neural network stability assessed via fMRI, which we hypothesize will improve with supplementation and dietary changes.*
Tertiary/Exploratory		
As post hoc and only exploratory analyses, we will regress differences pre- and post-intervention in various mood/emotional state, global functioning, and laboratory/mechanistic metrics.	Neural uptake of ketone bodies as measured via MRS Glucose lability (glucose spikes demonstrated via CGM trends) Glucose Ketone Index (GKI) (as measured via Keto-Mojo device) Mood instability as ascertained via self-reported mood surveys Metabolic biomarkers (e.g., hs-CRP or serum lactate)	**Exploratory Hypothesis 1**: Greater neural uptake of ketone bodies as measured via MRS will correlate with greater improvement in neural network stability. **Exploratory Hypothesis 2**: Greater glucose lability (glucose spikes demonstrated via CGM trends) will negatively correlate with neural network stability. **Exploratory Hypothesis 3**: Lower Glucose Ketone Index (GKI) (as measured via Keto-Mojo device) will positively correlate with neural network stability **Exploratory Hypothesis 4**: Neural network instability as ascertained via fMRI may correlate with mood instability as ascertained via self-reported emotional state surveys. **Exploratory Hypothesis 5**: Ketone ester supplementation combined with low glycemic index dietary changes may correlate with improved metabolic biomarkers (e.g., hs-CRP or serum lactate), reflecting anti-inflammatory and pro-mitochondrial mechanisms. **Exploratory Hypothesis 6**: Greater neural uptake of ketone bodies as measured via MRS and greater increases in blood ketone level as measured via Keto-Mojo device may correlate with greater improvements in global functioning measures.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Study Design: General Overview

In designing a pilot study, it is important to consider the distinction between a strict ketogenic diet and exogenous ketones, the latter of which can be consumed in a supplementary manner while maintaining a regular or modified diet. Clinical benefits derived from the ketogenic diet are likely attributable to a combination of low glycemic index and the direct (and indirect) metabolic/signaling effects of ketone bodies themselves. However, the proportional contribution from each of these components is not fully understood and likely varies based on the clinical setting. While a strict ketogenic diet encapsulates the entirety of these potential contributory components, it also sets a high bar for adherence in a population that already struggles with treatment adherence. In order to optimize ease of adherence, we propose supplementation of exogenous ketone esters combined with a low glycemic index diet for this pilot study, which will track clinical outcomes and global functioning metrics alongside metabolic markers. Moreover, given the stabilizing effects on neural networks demonstrated with exogenous ketone ester supplementation, the protocol features collection of baseline and follow-up fMRI data to assess neural network stability before and after the intervention. Combining clinical assessments, metabolic markers, and functional neuroimaging data into a multifaceted analysis will provide a comprehensive window into the dysregulation underlying bipolar disorder and the mechanisms by which this metabolic intervention operates.

The overarching goal of this open-label, exploratory pilot study is to explore biomechanistic effects of exogenous ketone supplementation combined with a low glycemic diet (in effect, a 'ketogenic-mimicking diet') for patients with bipolar disorder. This represents a less restrictive metabolic intervention compared to a strict ketogenic diet while replicating two foundational elements of the ketogenic diet: low levels of glycemic/insulin signaling and high levels of circulating ketones. In addition to assessments of mood stability and global functioning, we will obtain functional neuroimaging data to better characterize the biomechanistic effects of this intervention.

Primary Aim: The primary aim of this biomechanistic pilot study (conducted in n = 12 individuals with bipolar disorder) is to assess if a low glycemic index dietary intervention combined with open-label supplementation of exogenous ketones, namely bis hexanoyl (R)-1,3-butanediol (available over the counter as an athletic performance and health supplement called Qitone 'Pro-Ketone' powder and also referred to as 'C8 ketone di-ester') can mimic two fundamental mechanisms commonly associated with ketogenic diets: namely, increases in blood beta-hydroxybutyrate levels and decreases in blood glucose lability. While a comprehensive low glycemic index diet may also be considered to augment the effects of exogenous ketone supplementation, for simplicity and ease of adherence the low glycemic index intervention in this protocol will be comprised of four simple dietary changes as follows:

1. Not consuming soda for the duration of the intervention period (diet soda is excluded as well).
2. Not consuming candy/sweets for the duration of the intervention period.
3. Not consuming white grains/rice for the duration of the intervention period, instead replacing these with complex carbohydrates such as whole grains and brown rice.

4. If consuming fruit with a meal, eating the fruit at the end of the meal to minimize glycemic spiking.

Timetable: This study, to be completed over a 3-year period, will include a net total of *n* = 12 subjects (gross total of *n* = 20 to account for attrition) with bipolar disorder.

Design Summary: Exploratory, single-arm pilot study assessing open-label ketone ester supplementation (19 g BID PO; 12.5 g active ingredient per serving) in combination with low glycemic index dietary intervention.

In the event of an availability shortage of Qitone Pro-Ketone supplement, a separate supplement, called KetoneAid KE4 Pro Ketone Ester may be used as a temporary replacement. KetoneAid contains the same active ingredient as Qitone. If KetoneAid is used, participants will consume 20 g BID PO; 10 g active ingredient per serving) in combination with low glycemic index dietary intervention.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

No control group was incorporated for budgetary/feasibility purposes. However, data collection will include a baseline period of approximately 7-10 days week prior to starting the intervention, which will be followed by a post-intervention (i.e., 'washout') follow-up period of approximately 14 days (please refer to flowchart and timetable above for further details on this study design). This will allow for some intra-participant comparisons, with participants serving as their own controls in that regard.

4.3 JUSTIFICATION FOR INTERVENTION

The dosage selected for the ketone ester supplement was chosen in accordance with the recommended serving size and consumption directions provided by the manufacturer. This was informed by safety and tolerability research conducted prior to commercialization of the supplement. This particular supplement is produced in a cGMP-compliant facility that maintains high purity standards, which was a key factor in our selection process.

In regard to the length of the intervention, dietary changes are widely known to have less immediate effects when compared to other treatment modalities. For significant metabolic changes to occur from dietary changes, participants may need to maintain dietary changes for at least 3-4 months. As an example, the ketogenic diet trial in bipolar disorder recently completed at Stanford involved a 4-month intervention period. With these considerations in mind, this study protocol features a 3-month dietary intervention period.

4.4 END-OF-STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed the baseline assessments (both imaging and clinical), the mid-intervention assessment, and the post-intervention assessments (both imaging and clinical).

The end of study participation is defined as completion of the post-intervention follow-up assessment and device return shown in the protocol flow diagram (Sec. 1.2) as 'Follow-up Period' and timeline (Sec. 1.3) as 'Post-intervention Devices.'

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

1. 18 and older
2. Able to provide informed consent
3. Diagnosis of bipolar disorder, type I or II as determined primarily via medical/psychiatric documentation provided by Prechter bipolar research program or, alternatively if required for feasibility/budgetary purposes, via pre-existing medical/psychiatric documentation obtained from chart review or from mental health provider with patient consent
4. Regular access to smart phone capable of syncing biometric wearable data collection (specifically, compatible with Oura™ app)
5. If taking psychiatric medications, on stable regimen as defined by no medication changes for mood stabilizers and/or antipsychotics in prior 4 weeks

5.2 EXCLUSION CRITERIA

An individual may be excluded from participation in the study if any of the following criteria are met:

- Inability to adhere to dietary changes as specified in protocol (e.g., not in control of food selection)
- History of moderate-to-severe traumatic brain injury, e.g. loss of consciousness > 10 min, neurologic sequala
- Evidence of large vessel stroke or mass lesion on previous MRI or MRI obtained during study
- History of significant GI disease (e.g., malabsorptive disorder, gastric cancer, intestinal resection)
- Pregnancy (as determined via urine pregnancy test at study initiation), if capable of becoming pregnant, or breastfeeding
- Unwilling to utilize birth control method during course of study (e.g., barrier contraception, oral contraceptive, IUD), if capable of becoming pregnant
- Currently receiving treatment with insulin (e.g., chronic pancreatitis, diabetes mellitus)
- History of mitochondrial disorder and/or significant uncontrolled metabolic/medical disorder
- Active/current illicit substance use (and/or consumption of >1 alcoholic beverages per day) – defined as using psychoactive medications not as prescribed or using illicit substances (as determined via urine drug screen and screening interview)
- Use of marijuana or THC products more than once monthly on average
- Subjects with contra-indications to MR imaging, including pacemakers or severe claustrophobia, and/or size incompatible with scanner gantry, e. g., men over 6 feet tall that weigh more than 250 lbs, men under 6 feet tall that weigh over 220 lbs, women over 5'11" tall that weigh more

than 220 lbs, or women under 5'10" tall that weigh more than 200 lbs. Subjects of these weights or greater typically have difficult fitting into the fMRI scanner properly.

- Suicidal thoughts with plans or intentions, as assessed by C-SSRS
- Any other condition or criteria that would preclude safe and meaningful participation in this study

5.3 LIFESTYLE CONSIDERATIONS

During this study:

- Participants will be asked to take a ketone ester (KE) supplement twice daily.
- Participants will be asked to wear a continuous glucose monitor for approximately 7-10 days at baseline and at the end of the intervention period. They will also be asked to wear an Oura biometric ring throughout the intervention period.
- Participants may be required to fast in the morning prior to blood draw and/or abstain from exercise for at least several hours prior to blood draw.
- Glucose/ketone meter measurements will be performed at baseline, during the intervention (approximately mid-way at Day 45 ± 10 days), and at the end of the intervention period. Measurements will be taken before supplementation (morning fasting) and two hours post-supplementation.
- Participants will be asked to adhere to a low glycemic diet throughout KE intervention to minimize blood sugar spiking. Participants will be asked to follow a modified low-glycemic index diet in which they cut out soda/sugary drinks, candy, and white grains/rice. If consuming fruit, they will be asked to do so at the end of each meal to minimize glycemic spiking. If they desire more information, participants may be given a recipe sheet with off-limit foods/drinks and alternative meal/snack ideas.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in this study but are not subsequently assigned to the study intervention or entered in the study. Individuals who do not meet the criteria for participation in this trial (screen failure) because of meeting one or more exclusion criteria that are likely to change over time may be rescreened. Examples include the successful treatment of a previous affective disorder, and the lifting of dietary-related restrictions previously in place. Rescreened participants will be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment Sources

Potential participants will be recruited from several sources:

1) Primary recruitment source will be the Prechter Program Longitudinal Study of Bipolar Disorder

Other potential sources of recruitment include:

2) UMHealthresearch.org
3) Community advertisements
4) Referrals from the Prechter Program leadership and clinical research staff

Recruitment Strategy

Co-investigator Dr. Melvin McInnis oversees the Heinz C. Prechter Longitudinal Study of Bipolar Disorder, which actively follows over 900 patients with bipolar disorder, who are available for other research studies. As the participants in the Prechter study are used to being contacted for studies, they represent a ready source of potential participants. With IRB permission, letters and/or emails will be sent to Prechter participants letting them know about this study. If interested, they will be able to contact the study team, or learn more information through UMHealthresearch.org, which will maintain an entry for the study. Potential subjects will also be informed about the study by their treating clinicians. If they are interested in research activities, and screening of their records indicates they are suitable candidates, they will be sent a consent form and a follow-up phone call will be made by a study coordinator.

Participation in this study will have no direct effect on a subject's course of treatment.

Potential primary recruitment messages will consist of flyer/email template contents. In addition, the University of Michigan Clinical Translational Science Award, known as the Michigan Institute for Clinical & Health Research (MICHR), maintains UMhealthresearch.org -- >60,000 research volunteers registered -- which provides links to all studies going on in the medical center, conveniently linkable from social media campaigns, along with tracking metrics to determine the relative success of various search strategies. This web-based platform is extremely effective at providing an ongoing pool of subjects for research projects, both healthy control subjects as well as patient participants linked to specific studies.

Although individuals of other genders and ages (18 and older) are permitted to participate in our study, perimenopausal women (age 40-60) will be prioritized for recruitment in our sample given the historically common omission of this demographic from clinical research and resultant need for improved data to benefit women in this age range. Moreover, women in this age range are particularly prevalent in the Prechter Program Longitudinal Study of Bipolar Disorder, our primary recruitment channel. Every effort will be made to recruit at least 25% of participants representing minority groups (thus roughly approximating the heterogenous makeup of the American population at large).

To account for attrition, up to 20 participants may be recruited, with the goal of achieving a net study completion of 12 participants.

Recruitment Compensation & Retention Strategies

Participants will receive $50 for each of the clinical testing visits (total of $150 collectively for the pre-intervention, mid-intervention, and post-intervention visits).
Participants will receive $100 per completed PET or MRI scan (total of $300 collectively for imaging with breakdown as follows: $200 for first imaging visit, featuring both MRI and PET at $100 reimbursement each; $100 for second imaging visit, featuring only MRI).
Participants will receive a total of $50 for each week of using the continuous glucose monitor (CGM) – thus a combined total of $100 for the two weeks.
Participants will receive $200 for completing the approximately 90-day intervention of ketone ester supplementation combined with a low-glycemic index diet.

Participants will receive $50 for completing both the pre-intervention and post-intervention blood draws (thus $25 per blood draw).

If participants opt in for the optional PET scan and blood draws, compensation for their time and effort after full study completion may total a maximum of $800.

Participants will be paid after their last study visit or, in case they decide to withdraw from the study, they will be paid for the parts that they have completed. Participants will be paid by check which will be sent to their home address. Alternatively, participants may request a payment coupon for cash payment at the University Hospital. We do not keep cash for immediate payment.

Overnight accommodations may be provided depending on personal circumstances or if participants live far away. We will discuss with participants the need for these accommodations as the research appointment(s) are being arranged. If eligible, overnight lodging can be arranged through the UMHS Patient and Visitor Accommodations Program either by a study team member or by participants. However, participants may decide to make alternative arrangements. In that case, they will be instructed to discuss with the study team first if they are eligible for reimbursement prior to making any reservations. We can only reimburse for expenses that have been approved in advance by the study team. Participants will need to provide receipts to the study team before expenses can be reimbursed. We will reimburse to a maximum of $300 for lodging and meals.

Participants will receive a voucher for valet parking at the University Hospital. Parking at Domino's Farms is free.

6 STUDY INTERVENTION(S) OR EXPERIMENTAL MANIPULATION(S)

6.1 STUDY INTERVENTION(S) OR EXPERIMENTAL MANIPULATION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION OR EXPERIMENTAL MANIPULATION DESCRIPTION

The study intervention in this protocol will be referred to as a 'Ketogenic-mimicking Diet' – in summary, this involves dietary changes intended to minimize glycemic spiking combined with supplementation of exogenous ketones (agent/dosage specified below in 6.1.2). This combination thus mimics two foundational components of the ketogenic diet (namely, high levels of circulating ketones and low levels of blood glucose/insulin spiking). To optimize ease of adherence and feasibility for participants, the dietary changes will consist of four simple modifications as follows:

1. Not consuming soda for the duration of the intervention period.
2. Not consuming candy/sweets for the duration of the intervention period.
3. Not consuming white grains/rice for the duration of the intervention period, instead replacing these with complex carbohydrates such as whole grains and brown rice.
4. If consuming fruit with a meal, eating the fruit at the end of the meal to minimize glycemic spiking.

The exogenous ketone ester in this protocol, namely bis hexanoyl (R)-1,3-butanediol (available over the counter as an athletic performance and health supplement called Qitone 'Pro-Ketone' powder) will be provided via open-label supplementation. In the event of an availability shortage of Qitone Pro-Ketone supplement, a separate supplement, called KetoneAid Ke4 Pro Ketone Ester may be provided via open-label supplementation as a temporary replacement. KetoneAid contains the same active ingredient as Qitone. If KetoneAid is used, participants will consume 20 g BID PO; 10 g active ingredient per serving) in combination with low glycemic index dietary intervention.
For budgetary and feasibility purposes, no placebo control group will be included in this pilot study. Instead, participants will serve as their own controls with pre- and post-intervention data collection, and the protocol will include assessment of objective biomarkers (including hs-CRP, nocturnal HRV, and fMRI neural network stability) to account for potential placebo effects.

6.1.2 ADMINISTRATION AND/OR DOSING

The ketone ester in this study (available over the counter as Qitone 'Pro-Ketone' powder) will be consumed twice daily PO by mixing 19 g of the powder (12.5 g of active ingredient per serving) into water. If taking KetonAid, this ester will be consumed twice daily PO and will not require mixing (20 g Ketone Ester [Liquid]; 10 g active ingredient per serving). Participants will be advised to take the supplement upon waking, with the second dose occurring in the late afternoon or early evening (providing flexibility to account for varying work schedules). If necessary for tolerability (ketone esters have a naturally bitter flavor), participants will be advised that taste preferences can be accommodated with a touch of over-the-counter electrolyte powder (which neutralizes the naturally bitter flavor of ketone esters). The intervention will be consumed independently by participants after they receive consumption instructions in the lab setting prior to beginning the intervention period.

6.2 FIDELITY

6.2.1 INTERVENTIONIST TRAINING AND TRACKING

Study coordinators will monitor participant adherence with weekly phone call check-ins. Upon study completion, they will assess how much ketone ester supplement has been exhausted from containers to calculate approximate servings consumed.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

For budgetary and feasibility purposes, no placebo control group will be included in this pilot study. Instead, participants will serve as their own controls with pre- and post-intervention data collection, and the protocol will include assessment of objective biomarkers (including hs-CRP, nocturnal HRV, and fMRI neural network stability) to account for potential placebo effects.

6.4 STUDY INTERVENTION/EXPERIMENTAL MANIPULATION ADHERENCE

Adherence will be monitored via weekly phone call check-ins with study coordinators, adherence logs (i.e., daily check marks for supplement consumption and dietary adherence on a longitudinal sheet provided at study initiation [included in 'Additional Documentation' in Section 44.1]), FoodView meal diary smartphone app, CGM data during the final week of the intervention, and post-intervention supplement accountability assessment (including study coordinators measuring how much ketone ester supplement has been exhausted from containers at study completion).

6.5 CONCOMITANT THERAPY

For this protocol, participants will be maintained on the regular medications as prescribed by their physicians and other prescribers. They may also use over-the-counter (OTC) medications, although they will be discouraged from taking aspirin and acetaminophen due to the mitochondrial effects attributed to these OTC medications. For periodic control of pain, they will be directed to use ibuprofen. Medication usage will be assessed at each study visit. If a participant begins receiving insulin therapy for a comorbid condition during the course of the study, this may confound study results and metabolic outcomes – if deemed necessary after discussion with the PI, a participant who begins insulin therapy during the course of the study for a comorbid condition will in turn be disenrolled.

6.5.1 RESCUE THERAPY

If participants become unstable due to their underlying bipolar illness, symptom management will be done by their treating clinician. The study team will not be involved in any decisions about clinical management.

7 STUDY INTERVENTION/EXPERIMENTAL MANIPULATION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION/EXPERIMENTAL MANIPULATION

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

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

- The reason(s) for discontinuing the participant from the intervention, and methods for determining the need to discontinue
- If the participant is due to complete assessments within 4 weeks of being discontinued from the study intervention, those assessments may be administered at the time of discontinuation; if the next scheduled assessments are more than 4 weeks from the discontinuation date, the discontinued

participant may instead wait for the next scheduled assessment. Thereafter, the participant will be included in all future scheduled assessments, even though not participating in the intervention. Alternatively, if deemed appropriate by the PI, disenrollment in all study procedures may also be considered for participant safety, utility of collected data, or to conserve limited resources for those participants consistently adhering to the supplement/dietary changes.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue a participant from the study for the following reasons:

- Significant study intervention non-compliance, unless varying compliance is an aspect of the study objectives
- Lost-to-follow up; unable to contact subject (see **Section 7.3, Lost to Follow-Up**)
- Any event or medical condition or situation occurs such that continued collection of follow-up study data would not be in the best interest of the participant or might require an additional treatment that would confound the interpretation of the study
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Changes in the participants drug regimen for underlying bipolar disorder (except for use of as needed medications begun prior to study enrollment, and except for the addition of standard hypnotic agents used and discontinued with 5 days of neuroimaging studies).

The reason for participant discontinuation or withdrawal from the study will be recorded. Subjects that discontinue study participation prior to completion of all study visits may be replaced via recruitment of new subjects in order to obtain a minimum complete participation of 6 subjects.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 scheduled visits and study staff are unable to contact the participant after at least 3 attempts.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant, reschedule the missed visit within 21 days, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 ENDPOINT AND OTHER NON-SAFETY ASSESSMENTS

Study procedures and assessments:

1. Informed consent will be obtained prior to administration of any assessments, with the exceptions of the pre-screening questions (that subjects verbally agreed to answer in the recruitment phone call) and reviewing medical records (with a HIPAA waiver for recruitment). Questions will be answered during this time and participants will have an opportunity to get acquainted with the protocol prior to beginning the study. Prior to enrollment, an eligibility assessment visit will take place (which can be done virtually) to perform a suicidality screening, adverse childhood experience screening (ACE), and substance use history screening if applicable. An optional diagnostic confirmation interview (MINI, DIGS or SCID-V) administered by a trained clinician may also take place at the eligibility visit, but may be skipped to reduce participant burden if pre-existing diagnostic confirmation is available (e.g., if the participant already received diagnostic confirmation via the Longitudinal Study of Bipolar Disorder [HUM00000606]). Urine drug screening may be administered during the eligibility visit or , alternatively, at the baseline clinical/imaging visit prior to any imaging procedures.
2. After obtaining informed consent and screening for study eligibility, study participants will undergo the baseline clinical assessment and imaging protocol (fMRI and optional 18F-BCPP-EF PET) on separate days (in no particular order, allowing for some flexibility with potential scheduling hurdles). There will be a total of 5 visits for this pilot study, 2 of which will be completed before initiating the intervention. For the survey assessments, a trained clinician will administer the clinician-dependent surveys (e.g., YMRS, CGI, GAF, and C-SSRS assessments).
3. During the baseline imaging visit, participants will visit the radiology suite to complete the baseline MRI scan and optional PET scan. Magnetic resonance imaging (MRI) will be performed on a 3 Tesla Philips Achieva system (Philips, Best, The Netherlands). A 3D inversion recovery-prepared turbo-field-echo was performed in the sagittal plane using TR/TE/TI=9.8/4.6/1041ms; turbo factor=200; single average; FOV=240x200x160mm; acquired Matrix = 240x200x160 slices and reconstructed to 1mm isotropic resolution. Resting-state fMRI scans be acquired using a 32-channel head coil and multi-band sequence (Feinberg et al., 2013) [57], with nominal parameters: (TR/TE/FA = 720/34/52, 2mm isotropic resolution, 72 slices). Resting-state fMRI will be collected with participant's eyes fixated on a cross for 10 minutes} (Patriat et al., 2013) [58]. Magnetic resonance spectroscopy (MRS) may optionally complement the MRI sequence. MRS may be performed either at University Hospital or North Campus fMRI Laboratory. PET imaging will be performed in 3D imaging mode on a Biograph 6 TruePoint PET/CT scanner (Siemens Molecular Imaging, Inc., Knoxville, TN), which acquire 63 transaxial slices (slice thickness: 2.4 mm) over a 15.2 cm axial field-of-view. Imaging parameters described here are representative of what will be employed in the actual acquisitions and may differ slightly. MRI power-monitoring software on the scanner will ensure total energy delivered to the subject will remain within FDA guidelines.

Specifically, the specific absorption rate (SAR) will be not greater than: 1) 4 W/kg averaged over the whole body for any period of 15 minutes; 2) 3 W/kg averaged over the head for any period of 10 minutes; 3) 8 W/kg in any gram of tissue in the head or torso; 4) 12 W/kg in any gram of tissue in the extremities, for any period of 5 minutes.

4. If required for logistical purposes, participants may be instructed to start wearing a continuous glucose monitor (CGM) and a biometric device called Oura™ ring at the baseline imaging visit (with the logistical goal being to obtain baseline glycemic trends as well as sleep and activity data for 7 [±3] days prior to starting the intervention, while streamlining time commitment on behalf of participants). If so, the study coordinator will stop by the imaging suite to provide the CGM and Oura™ ring with the corresponding instructions (i.e., wearing the CGM for approximately 7-10 days and wearing the Oura™ ring throughout the day and night while charging it appropriately every 2-3 days).
5. The baseline exploratory clinical assessment (performed in-person by study coordinators and trained personnel) will include standardized assessments of global functioning and symptom severity (summarized in *Table 1*). Participants will also have their blood drawn for laboratory assessments at the pre-intervention clinical visit (assays detailed in *Table 2*)—blood-derived laboratory assays will be optional for participants (e.g., to accommodate participants with a strong preference to avoid blood draws or a specific phobia pertaining to blood). Upon completion of this baseline assessment, participants will initiate the 90 (±10)-day intervention (ketone ester supplementation in combination with low glycemic index dietary changes).
6. During the intervention period, participants will be prompted to submit daily ratings of mood via the CareEvolution platform. The daily mood ratings will be asked once daily at 7pm, and will involve brief subjective ratings of various emotional states and bodily feelings such as depressed mood, increased energy, fatigue, fidgetiness, and impulsivity. Participants will be able to click multiple choice options and adjust sliders to minimize time commitment for the surveys (free text is optional if desired and minimally featured). Study coordinators will provide weekly phone check-ins to monitor adherence and assess for adverse events between visits.
7. Participants will be asked to keep a food diary using via the FoodView platform during the intervention period. Tracking will involve using a smartphone to keep a photo-journal consisting of meals eaten each day during the intervention period. Study coordinators will provide weekly phone check-ins to monitor adherence.
8. Participants will also be instructed to check their blood ketone/glucose levels using a Keto-Mojo™ ketone/glucose monitor on intervention days 0 (baseline), 45 (±10) (mid-intervention), and 90 (±10) (post-intervention): measurements will be taken before supplementation (morning fasting) and two hours post-supplementation.
9. At the mid-intervention visit (which can be performed in-person or virtually via secure platform on day 45 ± 10 days), participants will repeat the comprehensive neurobehavioral assessment summarized in *Table 1* for reference.
10. At the end of the intervention period, participants will repeat a 7–10 day CGM assessment. They will repeat the clinical/laboratory assessment (performed in-person for the final clinical visit) and fMRI-only imaging protocol on separate days (as the optional PET scan is only performed at

baseline, the post-intervention imaging protocol involves only a repeat MRI scan as detailed in the imaging protocol above).

11. Participants will continue to wear their Oura™ ring throughout the intervention period and for a 14 (±7)-day post-intervention follow-up period. Daily mood ratings will also be continued during this post-intervention follow-up period until the Oura™ wearable is returned, at which point study participation will be completed. For a visual overview of this study design, please refer to Figure 1 (protocol flow chart) and Section 1.3 (schedule of activities chart).

During initial screening, participants will be screened prior to for the following to determine eligibility:

- History of moderate-to-severe traumatic brain injury
- Evidence of large vessel stroke or mass lesion on previous MRI or MRI obtained during study
- History of significant GI disease (e.g., malabsorptive disorder, gastric cancer, intestinal resection)
- Pregnancy (as determined via urine pregnancy test at study initiation), if capable of becoming pregnant, or breastfeeding
- Unwilling to utilize birth control method during course of study (e.g., barrier contraception, oral contraceptive, IUD), if capable of becoming pregnant
- Currently receiving treatment with insulin (e.g., chronic pancreatitis, diabetes mellitus)
- History of mitochondrial disorder and/or significant uncontrolled metabolic/medical disorder
- Active/current illicit substance use (and/or consumption of >1 alcoholic beverages per day) – defined as using psychoactive medications not as prescribed or using illicit substances (as determined via urine drug screen and screening interview)
- Use of marijuana or THC products more than once monthly on average
- Subjects with contra-indications to MR imaging, including pacemakers or severe claustrophobia

If a participant is deemed eligible and enrolls in the study, at baseline screening the following will be obtained:

- Blood draw for laboratory assays (e.g., hs-CRP, lactate, magnesium)
- Continuous glucose monitoring (to be placed by study coordinator and returned to the study team following 7-10 days of data tracking)
- Oura biometric ring sleep data tracking (to be worn by participant throughout the night and day, and charged every 1-3 days; participants will be blinded to their own data to avoid confounding effects)
- Neurobehavioral surveys (to be performed by trained individuals with prior clinical experience): detailed in the following table.

Table 1. Exploratory Neurobehavioral Survey Battery

Domain	**Metric**
Mania Symptomatology	Young Mania Rating Scale (YMRS)
Depression Symptomatology	Beck Depression Inventory (BDI) Patient Health Questionnaire-9 (PHQ-9) Columbia Suicide Severity Rating Scale (C-SSRS) Hamilton Depression Rating Scale (HDRS)

Mood Stability	Brief daily mood surveys delivered via CareEvolution platform
Global Functioning	Clinical Global Impression (CGI) Global Assessment of Functioning (GAF) Life Functioning Questionnaire (LFQ) Sheehan Disability Scale (SDS)
State and Trait Anxiety	Spielberger State-Trait Anxiety Inventory (STAI)
Quality of Life	36-Item Short Form Health Survey (SF-36)

These measures will be repeated at the post-intervention visit.

Self-reported measures include: BDI, PHQ-9, CareEvolution mood surveys, LFQ, SDS, STAI, and SF-36.

Rater-dependent measures (to be performed by trained personnel with clinical experience) include: YMRS, C-SSRS, CGI, HDRS, and GAF.

Survey-specific Details:

- Frequency/Timing: All of the following surveys will be administered at baseline then repeated at the mid-intervention and post-intervention visits.
- YMRS: The Young Mania Rating Scale (YMRS) is one of the most frequently utilized rating scales to assess manic symptoms. The items were selected based upon published descriptions of the core symptoms of mania. The YMRS followed the style of the Hamilton Rating Scale for Depression (HAM-D) with each item given a severity rating. Strengths of the YMRS include its brevity, widely accepted use, and ease of administration. See: Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry. 1978;133:429-435. doi:10.1192/bjp.133.5.429 [59].
- BDI: The Beck Depression Inventory (BDI) is a self-administered survey that assesses depressive symptoms and severity. See: Beck, A.T., R.A. Steer, and G.K. Brown, Manual for the Beck Depression Inventory-II. 1996, San Antonio, TX: Psychological Corporation [60].
- C-SSRS: The Columbia–Suicide Severity Rating Scale (C-SSRS) is a standardized screening tool to quantify the severity of suicidal ideation and behavior. See: Posner K, Brown GK, Stanley B, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *Am J Psychiatry*. 2011;168(12):1266-1277. doi:10.1176/appi.ajp.2011.10111704 [61].
- CGI: The Clinical Global Impression (CGI) was developed for use in NIMH-sponsored clinical trials to provide a brief, stand-alone assessment of the clinician's view of the patient's global functioning prior to and after initiating a study medication. See: Guy W, editor. ECDEU Assessment Manual for Psychopharmacology. Rockville, MD: US Department of Heath, Education, and Welfare Public Health Service Alcohol, Drug Abuse, and Mental Health Administration; 1976. [62].
- GAF: In 1962, the HSRS (Health-Sickness Rating Scale) was published. Studies of the HSRS resulted in a proposal for a new scoring system in the 1970s, the Global Assessment Scale (GAS). Further development led to Global Assessment of Functioning (GAF) in 1987. Today, the GAF

scale is well known internationally and widely used for scoring the severity of illness in psychiatry. It serves as a clinician-administered assessment that provides a scored global impression of functioning for individuals in the context of their clinical conditions.

- LFQ: The Life Functioning Questionnaire (LFQ) is a self-administered survey that assesses global functioning for participants. See: Altshuler L, Mintz J, Leight K. The Life Functioning Questionnaire (LFQ): a brief, gender-neutral scale assessing functional outcome. *Psychiatry Res*. 2002;112(2):161-182. doi:10.1016/s0165-1781(02)00180-4 [63].
- SDS: The Sheehan Disability Scale (SDS) is a self-administered survey that assesses the extent of disability and impairment experienced by individuals as a result of their symptoms. See: Arbuckle R, Frye MA, Brecher M, et al. The psychometric validation of the Sheehan Disability Scale (SDS) in patients with bipolar disorder. *Psychiatry Res*. 2009;165(1-2):163-174. doi:10.1016/j.psychres.2007.11.018 [64].
- STAI: The Spielberger State-Trait Anxiety Inventory (STAI) is a self-administered survey that assesses the severity of anxiety parameters, delineated by state (present feelings) vs. traits (general feelings). See: Spielberger CD, Gorsuch RL, Lushene RR. STAI Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press, Inc; 1970 [65].
- SF-36: The 36-Item Short Form Health Survey (SF-36) is a self-administered survey that assesses quality of life for individuals. See: Brazier JE, Harper R, Jones NM, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *BMJ*. 1992;305(6846):160-164. doi:10.1136/bmj.305.6846.160 [66].
- HDRS: The HDRS (also known as the Ham-D) is the most widely used clinician-administered depression assessment scale. See: Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960; 23:56–62 [67]

Table 2. Laboratory Assessments*

High-sensitivity C-reactive protein (hs-CRP)
Magnesium level (serum or RBC)
25-hydroxy vitamin D level
Peripheral mitochondrial function assessment (e.g., serum lactate, WBC mitochondrial DNA copy number, mitochondrial haplotype, or Seahorse assay)
Urine drug screen (UDS)

*Lithium level monitoring may be considered for participants taking lithium due to theoretical interaction effects in the setting of ketone metabolism.

8.2 SAFETY ASSESSMENTS

A pregnancy test will be used at the beginning of the study to exclude pregnant women from participating in the study. Women actively breastfeeding (as determined via initial screening) will also be excluded.

Unsolicited AEs will be captured by asking “Have you noticed anything different since you started the study or anything that concerns you about the intervention?” at each clinical visit as well as during phone check-ins. Solicited AEs will be captured by regularly screening for the following adverse events at each clinical visit (detailed below in patient-friendly language):

- Ketone ester supplements are usually well tolerated. However, possible infrequent side effects may include mild nausea, diarrhea, indigestion, upset stomach, distended abdomen, feeling bloated, heartburn, loose bowel movements, constipation, passing gas, keto flu-like symptoms, headache, dizziness, or sleep changes. Ketones may affect blood glucose levels (decrease or increase), which could lead to symptoms of lightheadedness or jitteriness. You may also feel more mental or physical energy with supplementation. Theoretically, increased energy availability provided by ketones may be a risk factor for (hypo)mania (which, in turn, is a risk factor for social consequences and impulsive behaviors such as impulsive spending, risk-taking, and sexual promiscuity), though we are not aware of any clinical reports that validate the possibility of hypomania induced by consumption of exogenous ketone supplements to date. It is possible that blood laboratory assay values may change while taking the ketone ester. For example, thyroid function tests may change, as the body interprets ketones as a signal for fasting (and, in turn, slowing down metabolism). It is theoretically possible that kidney function values may change, as the ketone ester will be excreted via the kidneys. If any concerning changes in blood laboratory assay values are identified during the course of the study, we may pause or discontinue the study to allow for any necessary medical attention for participant safety. Lastly, the ketone ester may have a poor or bitter taste, but the drink can be diluted.

Safety will be monitored at every visit and during weekly phone call check-ins with study coordinators. The SAFTEE screen will be utilized to assess for symptoms and quantify severity. Study coordinators will screen for any potential concerns or side effects then refer participants to clinicians if screening is concerning for a rating of 3 or higher (i.e. 'moderately' bothersome or more severe) for any SAFTEE symptom measures or any of the following:

- Keto flu-like symptoms
- Sleep disruption to point of causing participant distress or impairment
- Increased energy or symptoms of hypomania (such as impulsive spending, increased risk-taking, decreased need for sleep)

The Columbia Suicide Severity Rating Scale (CSSRS) will be performed at every clinical visit to monitor for active suicidal ideation during the course of the study. During each clinical visit, a clinician and/or medical student/study coordinator under clinician supervision will elicit any participant concerns for side effects or mood disturbance with the SAFTEE screening form and mood rating scales detailed above (i.e., BDI, YMRS, and C-SSRS). If concerns are identified at any point, one of the overseeing physicians will determine the correct course of action to ensure participant safety, which may include immediate cessation of ketone ester supplementation, discontinuation of study participation, and/or initiating suicide safety plans as necessary.

Specifically, for risk of suicidality (e.g., if a participant reports suicidal ideation as a free response in the 'Other' category while completing mood tracking on the CareEvolution app or refers to suicidal ideation during a weekly check-in with a study coordinator; if a participant selects answer responses 2 or 3 for question 9 on the Beck Depression Inventory, i.e., 'I would like to kill myself' [answer 2] or 'I would like to kill myself if I had a chance' [answer 3]; if a participant exhibits positive screening via C-SSRS – endorsement of suicidal thoughts or more), the full assessment on the C-SSRS will be completed to ascertain the level and severity of suicidal thoughts and behaviors. Dr. McInnis, Dr. Bohnen, and/or a designated clinician with psychiatric expertise will be contacted, and may discuss this with the participant. A conversation with the clinician must occur during that visit (either in-person or via phone/zoom) if the

patient expresses recent suicidal behavior (since last visit), or any past or current thoughts of self-harm (more than responding 'yes' to Q1 on the C-SSRS). The clinician will formulate the appropriate steps before the patient can leave the clinic, which may involve a safety plan with contact numbers or may require a trip to the emergency room for evaluation of high suicide risk. Documentation will be filed in the research record of the assessments and plan of action.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS

This protocol defines adverse events as: any untoward medical occurrence associated with the use of an intervention in humans, *deemed to be due to the intervention as determined by clinician review.*

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS

This protocol defines serious adverse events as: any untoward medical occurrence associated with the use of an intervention in humans that results in inpatient hospitalization or permanent disability, and is deemed to be due to the intervention as determined by clinician review.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

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

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the investigative measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION/EXPERIMENTAL MANIPULATION

All adverse events (AEs) will have their relationship to study procedures, including the intervention, assessed by an appropriately-trained clinician based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study procedures administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study procedures should be clinically plausible. The event must be pharmacologically or phenomenologically definitive.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study procedures, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of study procedures). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study procedures administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study procedures) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study procedures administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 EXPECTEDNESS

A clinician with appropriate expertise in ketogenic interventions will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study procedures.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

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

All AEs, not otherwise precluded per the protocol, will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study procedures (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring during the intervention will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical or psychiatric condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study in a manner deemed by designated clinician(s) to be related to the intervention, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Documentation of onset and duration of each episode will be maintained for AEs characterized as intermittent.

Nicolaas Bohnen, MD, PhD will record events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 14 days (for SAEs) after the last day of study participation. At each study visit, the study coordinator(s) will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

Jeff Bohnen, Melvin McInnis, MD, FRCPsych, and Nicolaas Bohnen, MD, PhD will act as safety monitoring members for this study. All adverse events and other study-related events will be discussed at weekly lab meetings (to be held as often as possible pending holiday/travel plans). Adverse events will be classified according to standard criteria and reported to the IRBMED (relevant University of Michigan IRB) according to standard guidelines. Serious adverse events will be reported no later than 10 working days after the investigator first learns of the event.

Disease-related events in the setting of bipolar disorder may include spontaneous depressive or (hypo)manic episodes not related to the intervention, which may lead to potential consequences such as impulsive spending/risk-taking, sexual promiscuity, depressed mood, or suicidality. If disease-related events are captured during regular monitoring (e.g., weekly phone check-ins, clinical visits, clinician interviews/surveys) they will be recorded, and relatedness to the intervention will be assessed as described above. This may result in safety planning for disease-related events unrelated to the intervention, such as referring a participant to the Psychiatric Emergency Service if deemed necessary.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

In consultation with the PI and faculty supervisors, a trained member of the study team will be responsible for conducting an evaluation of a serious adverse event and shall report the results of such evaluation to the Institutional Review Board (IRB) as soon as possible, but in no event later than 10 working days after the investigator first learns of the event.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Participants will be informed about AEs and SAEs in a face-to-face manner by the principal investigator and/or physician co-investigators. Incidental findings observed in blood work will be disclosed to

participants in a similar manner, with referral to primary care physician as necessary for any findings deemed concerning by clinicians.

8.3.8 REPORTING OF PREGNANCY

A urine pregnancy test will be performed prior to imaging. A positive test will result in disqualification for study participation.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS

This protocol uses the definition of Unanticipated Problems as defined by the Office for Human Research Protections (OHRP). OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEMS REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number
- A detailed description of the event, incident, experience, or outcome
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB within 10 working days of the investigator becoming aware of the event
- Any other UP will be reported to the IRB within 21 working days of the investigator becoming aware of the problem

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

If deemed necessary by the principal investigator, participants will be informed about UPs on an individual basis in a face-to-face manner.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Endpoint(s):

We hypothesize that: blood beta-hydroxybutyrate level will increase significantly with ketone ester supplementation combined with low glycemic index dietary changes.

- Secondary Endpoint(s):

We hypothesize that: blood glucose lability will decrease significantly with ketone ester supplementation combined with low glycemic index dietary changes.
We hypothesize that: DMN neural network stability derived from fMRI (defined by correlations and anticorrelations among brain regions over time as based on calculations originated by Mujica-Parodi et al. [68]) will improve with ketone ester supplementation combined with low glycemic index dietary changes (as compared to DMN neural network stability measured at baseline).

As *post hoc* and only exploratory analyses, we will regress differences pre- and post-intervention in various mood, global functioning, and biomechanistic laboratory outcome measures.

9.2 SAMPLE SIZE DETERMINATION

For budget and feasibility purposes, net sample size for this pilot study will be 12 participants (gross sample size of n = 20 to account for attrition), which will be sufficient to gauge if ketone ester supplementation combined with low glycemic index dietary changes has the potential to mimic mechanistic changes classically associated with strict ketogenic diets and to collect qualitative feedback for implementation strategies. We do not necessarily expect statistically significant effects to be observed for mood or biomarker endpoints, and will assess these as exploratory outcomes only.

9.3 STATISTICAL ANALYSES

9.3.1 GENERAL APPROACH

For descriptive statistics, categorical and continuous data will be presented by reporting percentages, means with standard deviations, medians, and ranges.

For qualitative research, procedural and interpretive rigor will be maintained by using semi-structured interview approaches with a standardized set of questions to explore qualitative dimensions of intervention adherence (i.e., focused on strengths, weaknesses, and areas of improvements for feasibility of the lifestyle intervention). In addition, Likert scale assessment (1-5 rating scale ranging from "strongly disagree" to "strongly agree) for the statement "I was able to achieve at least 70% adherence to this regimen (supplement combined with moderation of sugar intake) for 3 months," will be obtained to further standardize qualitative responses.

As *post hoc* and only exploratory analyses, we will regress differences pre- and post-intervention in various mood/clinical, global functioning, and laboratory outcome measures.

9.3.2 ANALYSIS OF THE PRIMARY ENDPOINT(S)

Keto-Mojo devices will be used to measure blood beta-hydroxybutyrate levels at baseline and after taking the supplement to assess both immediate and sustained effects on blood beta-hydroxybutyrate (for complete schedule of measurements please refer to Section 1.3). Measurements taken at baseline, mid-intervention, and post-intervention will be compared and regressed to analyze associated changes.

9.3.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

CGM devices will be used to capture blood glucose lability, defined as frequency of glycemic spiking averaged per day. Baseline blood glucose lability and post-intervention blood glucose lability will be compared to analyze associated changes. For neural network stability, fMRI will be used to measure DMN-specific neural network stability (defined by correlations and anticorrelations among brain regions over time as based on calculations originated by Mujica-Parodi et al. [68]). Computed neural network stability after the supplementation/dietary change period will be compared to computed neural network stability at baseline.

9.3.4 EXPLORATORY ANALYSES

As *post hoc* and only exploratory analyses, we will regress differences pre- and post-intervention in various mood/clinical, global functioning, and laboratory outcome measures. Exploratory analyses will include the following elements:

- MRS ketone body brain uptake pre- and post-intervention
- Mitochondrial complex I PET ligand regional uptake at baseline as a predictor of changes in the fMRI neural network stability measure
- Changes in hs-CRP, lactate, and peripheral mitochondrial biomarker measures pre- vs. post-intervention

- Changes in mood stability during the intervention period (as determined via CareEvolution secure platform daily surveys) and correlations with neural network stability findings
- Changes in blood glucose trends (obtained via CGM) pre- vs. post-intervention
- Changes in blood ketone levels during the intervention period

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks will be given to the participant and written documentation of informed consent will be completed prior to starting the study intervention. The following consent materials are submitted with this protocol: informed consent form, recruitment email and flyer templates, and phone screening script.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Subjects will first be informed of all aspects of this project in a face-to-face visit if recruited from the Prechter Program at the University of Michigan. Alternatively, subjects may be recruited from the Michigan Institute for Clinical & Health Science Research, in which case online materials and phone screenings will provide an introduction to this project for potential participants. Community advertisements may also be employed as a potential recruitment modality if required for feasibility purposes. When contacted by phone, the pre-screening questionnaire will be used. The study will be thoroughly explained and all questions will be answered. Verbal consent will be documented in screening. A copy of the informed consent will be mailed to people who are recruited by phone prior to the first study visit so potential participants have a chance to read it. Subjects will follow up with the study team to schedule their first visit.

In a face-to-face meeting, usually with the study coordinator, the study will again be explained and all questions will be answered. Subjects will provide written informed consent using documents approved by the Institutional Review Board. Electronic informed consent may be utilized (via SignNow) to reduce face-to-face interactions. The subject will be reminded that participation is voluntary, and at any time the study may be stopped by the volunteer. He/she may ask questions at any time. The study coordinator will state that she is willing to answer questions at any time, and follow-up calls from research subjects are welcome and appreciated. The subject will be given a copy of the consent document and asked to keep it for reference as it contains contact names and numbers. A narrative of the informed consent process will be documented.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding sources, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor/funding agency and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance of study staff to the protocol (i.e., significant protocol violations)
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the funding agency, sponsor, IRB, Food and Drug Administration (FDA), or other relevant regulatory or oversight bodies (OHRP, DSMB).]

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, the safety and oversight monitor(s), and the sponsor(s) and funding agency. This confidentiality is extended to the data being collected as part of this study. Data that could be used to identify a specific study participant will be held in strict confidence within the research team. All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor or funding agency, or representatives of the Institutional Review Board (IRB) may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor/funding agency requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Functional Neuroimaging, Cognitive, and Mobility Laboratory. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of

the study, all the hard-copy research records will be stored for at least 10 years in the Functional Neuroimaging, Cognitive and Mobility Laboratory in a locked and limited-access storage room. Subject workbooks will also be stored electronically using Drobox for remote monitoring purposes. Image data files are maintained by Radiology and Nuclear Medicine in restricted access databases, not accessible via Internet or other outside connection.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

The intent and purpose of retaining research data is detailed in the informed consent form. Explicit approval will be sought from the subject for retention and/or data sharing. A data use agreement and/or material transfer agreement will be established first before research data sets are shared with external investigators.

Fully de-identified data sets may be shared with other investigators worldwide. Storage conditions of these data sets will be determined as part of the data use agreement and/or material transfer agreement.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Provide the name and contact information of the Principal Investigator and the Medical Monitor or Independent Safety Monitor. Update table heading to remove non-relevant role.

Principal Investigator	**Co-investigator**	**Faculty Supervisor**
Nico Bohnen, MD, PhD	*Jeff Bohnen, BSc, MD/MBA Candidate (2024)*	*Melvin McInnis, MD, FRCPysch*
Univ of Michigan	*Univ of Michigan*	*Univ of Michigan*
734 846 1748	*734 717 6989*	*734 355 8803*
nbohnen@umich.edu	*jbohnen@umich.edu*	*mmcinnis@med.umich.edu*

10.1.6 SAFETY OVERSIGHT

Jeff Bohnen, Melvin McInnis, MD, FRCPsych, a designated clinician with psychiatric experience, and Nicolaas Bohnen, MD, PhD will act as Data Safety Monitoring Plan members for this study. All adverse events and other study-related events will be discussed at monthly meetings. Adverse events will be classified according to standard criteria and reported to the IRBMED (relevant University of Michigan IRB) according to standard guidelines. Serious adverse events will be reported no later than 10 working days from the date an investigator becomes aware of a serious adverse event.

10.1.7 CLINICAL MONITORING

Clinical site monitoring will be conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Council on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). Monitoring activities will be conducted in accordance with University of Michigan IRB regulations.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical staff will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented as follows:

Informed consent --- Study staff will review both the documentation of the consenting process as well as a percentage of the completed consent documents. This review will evaluate compliance with GCP, accuracy, and completeness.

Source documents and the electronic data --- Data will be initially captured on source documents (see **Section 10.1.9, Data Handling and Record Keeping**) and will ultimately be entered into the study database. To ensure accuracy, site staff will compare a representative sample of source data against the database, targeting key data points in that review.

Intervention Fidelity — Consistent delivery of the study interventions will be monitored throughout the intervention phase of the study. Procedures for ensuring fidelity of intervention delivery are described in **Section 6.2.1, Interventionist Training and Tracking**.

Protocol Deviations – The study team will review protocol deviations on an ongoing basis and will implement corrective actions when the quantity or nature of deviations are deemed to be at a level of concern.

Should independent monitoring be planned, the PI will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor/funding agency, and inspection by local and regulatory authorities.]

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection will be the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will be completed in an organized manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant consented/enrolled in the study. Data derived from source documents recorded electronically will be consistent with the data recorded on the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents will be retained for a minimum of 2 years after study completion.

10.1.10 PROTOCOL DEVIATIONS

This protocol defines a protocol deviation as any noncompliance with the clinical trial protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- Section 4.5 Compliance with Protocol, subsections 4.5.1, 4.5.2, and 4.5.3
- Section 5.1 Quality Assurance and Quality Control, subsection 5.1.1
- Section 5.20 Noncompliance, subsections 5.20.1, and 5.20.2.

It will be the responsibility of the site investigator to use continuous vigilance to identify and report deviations per IRBMED policies. All deviations will be addressed in study source documents and reported to the IRB per University of Michigan policies.

10.1.11 PUBLICATION AND DATA SHARING POLICY

Fully de-identified data sets may be shared with other investigators worldwide. Storage conditions of these data sets will be determined as part of the data use agreement and/or material transfer agreement.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in

conjunction with the University of Michigan has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS AND SPECIAL TERMS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list). Special terms are those terms used in a specific way in the protocol. For instance, if the protocol has therapist-participants and patient-participants, those terms could be included here for purposes of consistency and specificity.

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Council on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities

MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.3 PROTOCOL AMENDMENT HISTORY

*The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A **Summary of Changes** table for the current amendment is located in the **Protocol Title Page**.*

Version	Date	Description of Change	Brief Rationale

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