Brain Small Chain Fatty Acid Metabolism in Parkinson Disease: Tributyrin Supplementation

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CLINICAL PROTOCOL

STUDY TITLE

Brain Small Chain Fatty Acid Metabolism in Parkinson Disease: Tributyrin Supplementation

Study Agents: Tributyrin; [^{11}C]Butyrate; [^{18}F]FDG

University of Michigan IRB HUM #00211320

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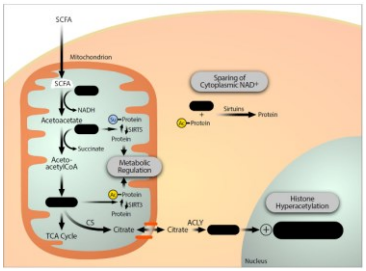
Brain Small Chain Fatty Acid Metabolism in Parkinson's Disease: Tributyrin supplementation

Cognitive decline, progressing usually to dementia, is a common and disabling feature of Parkinson's disease (PD). Progression to dementia generally reflects progressive cortical pathologies. We and others showed that cognitive decline is accompanied by and heralded by deficits in regional neocortical glucose metabolism, demonstrated with [^{18}F]fluorodeoxyglucose (FDG) PET imaging [1, 2]. These deficits are widely believed to reflect synaptic loss. Convergent imaging data, however, suggests relative preservation of neocortical synapses with abnormal neuronal glucose metabolism. Recent studies of PD and Alzheimer disease (AD) subjects with synaptic terminal SV2A PET ligands indicate preservation of cortical synaptic terminals at early to moderate disease stages. The major alternative brain energy substrate is ketone bodies that are small chain fatty acids (SCFAs). Comparative studies of brain SCFA and glucose uptake in Mild Cognitive Impairment and early AD subjects indicates relative preservation of regional brain SFCA metabolism in subjects with abnormal regional glucose uptake, suggesting relative preservation of synapses.
This accumulated PET data is broadly consistent with the concept that mitochondrial dysfunction is a major feature of, and likely pathogenetic mechanism in, PD. Other data suggests relevant dysfunction of glucose uptake and metabolism mechanisms in PD. One implication is that enhancing neuronal SCFA metabolism will circumvent abnormalities of glucose uptake and initial metabolism, enhancing-restoring neuronal mitochondrial function. This would potentially provide symptomatic benefits and might be a disease-modifying therapy (**Figure 1**). There are other good reasons to pursue SCFA therapy in PD. SFCAs may be significant modulators of inflammatory, oxidative, and transcriptional regulatory processes linked to neurodegeneration in PD (**Figure 1**). Recent experimental work suggests important links between mitochondrial dysfunction and pathogenic inflammatory mechanisms in PD. SCFA therapy may address the brain-gut axis dysbiosis that seems to characterize PD.



Capitalizing on our experience with PD clinical research and development of novel PET methods, we propose an integrated series of experiments to measure brain SCFA metabolism and to assess target engagement of SCFA therapy in older adults and in persons with Parkinson disease (PD).

Aim 1: To explore [^{11}C]butyrate PET and [^{18}F]FDG PET glucose metabolism and clinical measures before and after open-label treatment with the SCFA prodrug tributyrin in a small pilot study in patients with PD (n=8) and normal control older adults (n=2). A separate subset of 10 participants may complete the study without the PET or brain imaging biomarkers but using the exploratory clinical or laboratory test battery only for explorative reasons.

Hypothesis 2: [^{11}C]butyrate PET and glucose metabolism may change in the brain, liver, heart and gut after approximately 30-day intervention with tributyrin 500 mg t.i.d. p.o. with meals in patients with PD and normal older adults.

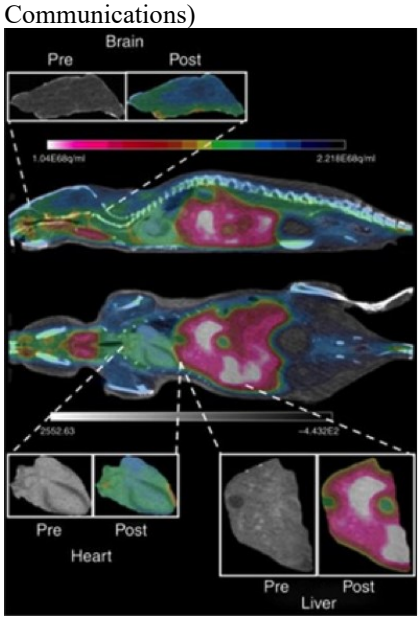
Exploratory hypothesis: Tributyrin supplementation may affect measures of cognition, autonomic, sleep metabolic, genetic, and other measures.

Impact: Positive findings in this small exploratory pilot trial may support future target engagement studies of SCFA supplementation in normal adults and PD.

Background

Ketogenic diet and SCFA interventions in neurodegeneration:
The ketogenic diet based on preferential intake of predominant medium and long-chain fatty acids and minimization of carbohydrates has become a subject of recent research in metabolic and neurodegenerative disorders. Preliminary positive findings have been reported mainly from short-duration studies. Despite the benefits of this diet, health care professionals still question its safety due to the elevated serum ketones it induces and the limited dietary fiber intake [6]. To compound the controversy, patient compliance with the program is poor due to the restrictive nature of the diet and symptoms related to energy deficit and gastrointestinal adversity during the introductory and energy substrate transition phase of the diet. Another class of fatty acids, so-called short chain fatty acid (SFCA), has been extensively studied for their role in the gut microbiome. For example SCFAs, in particular n-butyric acid (BA), acetic acid and propionic acid, are naturally present in the colon as a bacterial product of dietary fiber fermentation. Renewed interest in SCFAs has also emerged because of their ability to alter gene expression through histone deacetylase (HDAC) inhibition. HDAC has become a drug target in cancer as well as non-oncological diseases. These carboxylic acids are also of interest for their effects in the brain including memory enhancement and neuroprotection in rodent experiments [8]. Animal and human ingestion studies of prebiotic fibers associated with brain health, such as inulin and resistant potato starch, found selective increases in the production of colonic BA and acetic acid [10, 11]. Similarly, intermittent fasting in MPTP-induced Parkinson's disease mice resulted in higher butyric acid as well as increased levels of BDNF [13]. Although blood (and brain) levels of BA and acetic acid are naturally low [14], a ^{11}C-acetate PET study in rats found significantly increased uptake in the brain (as well as heart and liver) after 8 weeks of inulin fiber supplemented feeding, see Figure 1 [15]. Effects of BA on the brain may be direct or indirect. Indirect actions of butyrate are believed to involve the hormono–neuro–immuno system. For example, increased SCFA production in the colon may result in vagal nerve-mediated changes in the hypothalamus and induce blood-brain barrier changes. The 'gut brain axis' represents bidirectional communication between the gastrointestinal system and the brain and is based on a complex system, including the vagus nerve, sympathetic nerves, humoral, endocrine, and immune links. These observations suggest that SCFA may play a role in the so-called 'gut-brain axis' [19] and that interventions promoting SCFA may benefit patients with neurodegenerative or neuro-inflammatory disorders.

Figure 2: Brain, heart and liver uptake of ^{11}C-acetate before and after 8 weeks of inulin fiber supplemented feeding in rats (Frost et al. 2014 Nature Communications)



SCFAs can also be administered orally where gastric and duodenal absorption will result in increased portal vein and systemic plasma levels that may affect brain functions. For example, a ^{18}F-FDG PET study in pigs found that 3 weeks of oral ingestion of sodium butyrate triggered basal brain glucose metabolism changes in the nucleus accumbens and hippocampus with histological evidence of increased hippocampal granular cell layer volume and neurogenesis [21]. Intra-peritoneal administration of sodium butyrate in 6-OHDA-lesioned rats resulted in significant attenuation of motor deficits and increased striatal dopamine levels [22].

Moreover, sodium butyrate treatment attenuated oxidative stress and neuro-inflammatory markers. These effects occur concurrently with increased global H3 histone acetylation and BDNF levels [22].

BA and its prodrug tributyrin are naturally present in butter. In spite of its promise, systemic butyrate supplementation suffers from several limitations as butyrate is metabolized rapidly as soon as it enters the colonocyte via its active transport system [24] and resulting plasma (or brain) concentrations are very low. Tributyrin, a stable and rapidly absorbed prodrug of butyric acid offers several advantages compared to butyrate. Tributyrin is a neutral short-chain fatty acid triglyceride that is likely to overcome the pharmacokinetic drawbacks of natural butyrate as a drug [25]. Because it is rapidly absorbed and chemically stable in plasma, tributyrin diffuses through biological membranes and is metabolized by intracellular lipases, releasing therapeutically effective butyrate over time directly into the cell. Compared with butyrate, tributyrin has more favorable pharmacokinetics [25, 26] and is well tolerated [27]. Liquid tributyrin filled into gelatin capsules and administered orally resulted in millimolar concentrations of butyrate both in plasma and inside the cell [27]. Unlike BA, tributyrin is lipophilic with a plasma half-life of 40 minutes making it a preferred choice for systemic oral administration.

Overall strategy

Overview
The overarching goal of this small exploratory open-label pilot study is to explore [^{11}C]butyrate PET and [^{18}F]FDG PET glucose metabolism and clinical measures before and after open-label treatment with the SCFA prodrug tributyrin in a small pilot study in PD (n=8) and normal control older adults (n=2). Positive findings in this small exploratory pilot trial may support target engagement study of SCFA supplementation in normal adults and PD. PET and/or MRI Imaging protocols will be optional for a separate subset of 10 participants may also complete the study based on clinical or laboratory test battery alone for the exploratory hypothesis.

Timetable: This study, to be conducted over a 2-year period, will include a net total of *n=20 subjects (NC, n=4; PD, n=16; total net; n=20; gross n=32 to allow for attrition).* Details of the enrollment and major test procedures are listed in **table 1** below.

Milestone of recruitment success are net completion of 9 persons for year 1.

Table 1: Study enrollment & main assessments	**NC**	**PD**	**TOTAL**
Baseline [^{11}C]butyrate PET and [^{18}F]FDG PET-CT & brain MRI with baseline clinical assessment	NC=2	PD=8	n=10
Post-intervention [^{11}C]butyrate PET and [^{18}F]FDG PET-CT & MRI	NC=2	PD=8	n=10
30-day (± 7) tributyrin intervention with pre/post clinical assessments)	NC=4	PD=16	n=20
Exploratory clinical and laboratory test battery pre and post the tributyrin supplement intervention	NC=4	PD=16	N=20
Total subject net recruitment aims 1 & 2	NC=4	PD=16	n=20*

**Net* recruitment n=20; *gross* recruitment to account for attrition n=32

Design: Exploratory open-label tributyrin supplementation pilot study.

Methods: After obtaining informed consent and screening for study eligibility, study participants will undergo the baseline clinical assessment and imaging protocol (separate days). For patients with PD the motor component of the test battery will be performed in the dopaminergic medication 'off' state in the morning after overnight withholding of their dopaminergic medications in the PD, i.e., temporarily postponing the morning dose until the motor exam has completed. The remainder of the test battery will be performed in the medication 'on' state. Participants will also complete some testing in a fasted state. For participants who were not consented prior to baseline, study staff will obtain a waiver of consent during screening call for fasting and withholding dopaminergic medications prior to their visit. Qualified personnel who have been trained in the use of the instruments and have undergone inter-rater reliability evaluations will perform the assessments.

There will be a total of 5 visits for this pilot study. The first two visits will consist of the initial general clinical assessment (e.g., vital signs, vital capacity lung/breath holding tests) and optional imaging split over 2 days (no specific sequence or preset time interval). At visit 1 participants will be instructed to wear physical activity and sleep trackers (called ActivPal a sensor will be put on the upper leg and a ring called Oura ring, which will be worn at a finger) for 1 week prior to visit 2. Participants will also wear a continuous glucose monitor during the same time. After the participants complete the 1 week of physical activity, sleep and glucose monitoring they will return for visit 3, where a limited repeat clinical test battery will be administered. At visit 3 the participant will also be instructed to start the approximately 30 day supplementation of tributyrin. The physical activity and sleep trackers and the continuous glucose monitor will also be worn during the last week of the tributyrin supplementation period. At the end of the approximately 30-day tributyrin supplementation the participants will return for visits 4 (repeat clinical assessment) and 5 (repeat optional imaging) again split over 2 days. There is no pre-set sequence which assessment (clinical or imaging) comes first. Blood and/or saliva tests will be collected for assessments of genetic variables (e.g. ApoE). Blood assessment will also be used for metabolomics. Blood continuous glucose data will be collected blinded to the participant.

Details for the schedule of activities for the tributyrin supplement intervention study are shown below (Table 2).

Table 2 Schedule of activities	Visit 1* / Visit 2*: Baseline assessment (clinical)	Visit 1* / Visit 2* Baseline assessment (imaging – OPTIONAL)	At-home wearable sensor use (1 week ± 3 days)	Visit 3 Pre-intervention assessment	At-home 4 (± 1) week Tributyrin (500 mg tid po) intervention	Visit 4* / Visit 5* Post-intervention clinical assessment (while on tributyrin)	Visit 4* / Visit 5* Post-intervention imaging assessment (while on tributyrin, OPTIONAL)
Approximate time commitment	4-6 hours	3-6 hours	-	4-5 hours	-	4-6 hours	3- 6 hours
Test location	Dominos Farms	UM hospital	Home	Dominos Farms	Home	Dominos Farms	UM hospital
Informed Consent (or prior eConsent)	X						
Eligibility	X						

OPTIONAL [^{11}C]butyrate, [^{18}F]FDG PET-CT [1.2] (brain/body)*		X					X (after taking tributyrin)
In case of [^{11}C]butyrate PET, obtain vital signs pre- & post injection of tracer		X					X
OPTIONAL MRI brain*		X					X
DEXA Bone Density Scan	X					X	
Blood/saliva/urine sample collection[1]	X					X	
Continuous glucose monitor for 7±3 days			X		X (last 7 days)	X	
Sleep tracking ring			X	X	X	X	
Activity tracker for 7±3 days			X		X (last 7 days)		
Motor MDS UPDRS[2]	X			X		X	
Balance test MiniBESTest (sensored)[2]	X			X		X	
Pegboard testing[2]	X			X		X	
Foot Tapping[2]	X			X		X	
Finger Tapping[2]	X			X		X	
Electronic Gait Mat/sensored walk[2]	X			X		X	
Beck Depression Inventory II scale	X			X		X	
Spielberger Trait Anxiety Scale	X			X		X	
Sleepiness scales	X					X	
MoCa, PDCRS (limited cognition)	X			X (optional)		X	
Cognitive test battery	X			X (optional)		X	
PCFRS functional scale	X					X	
Quality of life scale PDQ-39	X					X	
Adverse event assessment	X			X		X	
Phone Call/Email Drug monitoring					X		
Drug accountability				X		X	X
Pregnancy test in women of		X		X			X

childbearing potential		(within 48 hours of PET)					(within 48 hours of PET)

Note
[1]: Fasting in morning
[2]: Dopaminergic off state in PD
*Visits 1-2 and 4-5. There is no pre-set sequence which assessment (clinical or imaging) comes first.

Details of the neuropsychological test battery is shown in Table 3.

Outcome measures:

Primary outcome measures:

- [^{11}C]butyrate uptake
- Change in average blood glucose metabolism measured by continuous glucose meter

Exploratory outcome measures:

- [^{18}F]FDG PET glucose metabolism
- Cognition dependent mobility functions: postural sway (iSWAY), Timed Up and Go test (iTUG) measured using Mobility Lab modules (APDM, Inc., Portland, OR) [28-30]
- Dynamic balance using the Mini-BESTest [31]
- Spatiotemporal gait measurements using Protokinetics Zeno™ Walkway (Protokinetics LLC, Havertown, PA)
- Remainder of the clinical and laboratory test battery

Table 3: Neuropsychological characterization

Overall mental status	Montreal Cognitive Assessment Scale [3] PD-Cognitive Rating Scale [4]
Intelligence	WAIS-IV: Information[5]
Attention & Cognitive Control	n-back test (OPTIONAL) [7], Stroop Color Word Interference Test [9] WAIS-IV: Digit Span (5) WAIS-III: Digit Symbol-Coding [5]
Language	Boston Naming Test (30 item) [12] Controlled Oral Word Association Test (Phonemic & Semantic)
Learning & Memory	California Verbal Learning Test – II [16]
Visuospatial/Construction	Clock drawing [17] Benton Judgment of Line Orientation [18]
Executive/Working Memory	D-KEFS: Color Word Test, Verbal Fluency Test, Trail Making Test, Sorting Test [20] WAIS-IV Matrix Reasoning, Digit Backwards [5]
Information processing speed	D-KEFS: Trail Making Test [20] WAIS-III: Digit Symbol-Coding [5, 23] Reaction time

Tributyrin intervention aim: BA and its prodrug tributyrin are naturally present in butter. In spite of its promise, systemic butyrate supplementation suffers from several limitations as butyrate is metabolized rapidly as soon as it enters the colonocyte via its active transport system [24] and resulting plasma (or brain) concentrations are very low. Tributyrin is a stable and rapidly absorbed prodrug of butyric acid offers several advantages compared to butyrate. Tributyrin is a neutral short-chain fatty acid triglyceride that is likely to overcome the pharmacokinetic drawbacks of natural butyrate as a drug [25]. Because it is rapidly absorbed and chemically stable in plasma, tributyrin diffuses through biological membranes and is metabolized by intracellular lipases, releasing therapeutically effective butyrate over time directly into the cell. Compared with butyrate, tributyrin has more favorable pharmacokinetics [25, 26] and is well tolerated [27]. Liquid tributyrin filled into gelatin capsules and administered orally resulted in millimolar concentrations of butyrate both in plasma and inside the cell [27]. Unlike BA, tributyrin is lipophilic with a plasma half-life of 40 minutes making it a preferred choice for systemic oral administration. Tributyrin is available over-the-counter as a supplement for patients with inflammatory bowel disease. Healus complete biotic butyrate supplement (500 mg tributyrin per

capsule) will be used as 1 capsule tid with meals (total of 1.5 g of tributyrin per day). If the patient cannot tolerate tributyrin we will lower the dose to one bid and if this is not tolerated than to once per day (e.g. at evening) and if this is not tolerated we will withdraw the study participant from the study. The participant will be asked to complete a daily drug diary while taking tributyrin. All supplement bottles will be returned at the end of the study for a drug accountability check.

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 will serve as a secondary outcome measure and be acquired using a 32-channel head coil and multi-band sequence [32], with nominal parameters: (TR/TE/FA = 720/34/52, 2mm isotropic resolution, 72 slices). Data are collected with eyes fixated on a cross for 10 minutes [33]. Pre-processing follows our standard pipeline that includes physiologic noise reduction and thorough motion correction [34, 35]. Graph theory analysis will be used to identify the most highly connected nodes following published methods. Briefly, ROIs will be represented as nodes and pairwise correlations, based upon the mean time series for each ROI, will serve as weights over corresponding edges, thresholded by FDR correction, $p<0.05$ [34, 35]. Change from baseline will serve as the primary measure of interest.

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. The i.v. injection dose for [^{11}C]butyrate PET is 18 mCi and for [^{18}F]FDG is 5 mCi. Vital signs will be obtained pre- and post-[^{11}C]butyrate injection. Images will be corrected for scatter and motion. The PET imaging frames will be spatially coregistered within subjects with a rigid-body transformation to reduce the effects of subject motion during the imaging session [36]. Statistical parametric mapping (SPM) software (SPM12; Wellcome Trust Centre for Neuroimaging, University College, London, England [https://www.fil.ion.ucl.ac.uk/spm/software/spm12/] will be used for PET-MRI registration using the cropped T1-weighted MR volumetric scan. Freesurfer software (http://surfer.nmr.mgh.harvard.edu) will be used to define cortical and subcortical volumes-of-interest (VOI).

MRI-based PET partial volume correction: We will use the Müller-Gärtner partial volume correction technique, which is based on the assumption that white matter uptake is homogeneous and uses gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) to correct the partial volume effect in GM tissues [37]. Using the segmented images and the assumed PET resolution, the method calculates the spill-out from WM to GM and subsequently subtracts it from the GM. Similarly, it also calculates the spill-out from the GM to the surrounding tissues and compensates for the difference to the GM. The result is a grey matter image with corrected activity values in all voxels.

Statistical analysis

We will use mixed linear modeling to assess for changes in butyrate and glucose metabolism before and after the tributyrin supplementation.

As a *post hoc* and only exploratory analyses we will regress differences in with tributyrin-induced changes of the various clinical and laboratory outcome measures.

Sample size estimation: This experiment is a first in human study of this drug for treatment of glucose changes in a neurodegenerative condition. There is a preliminary experiment of this safe drug conducted in high-fat fed mice that we base our same size justification off that showed mice receiving tributyrin showed a reduction in glucose levels compared to a comparator arm of approximately 13% with a standard deviation of 9% (PMID 22621868). If estimate a similar effect size in our cohort, estimating a mean plasma glucose of 110mg/dl at baseline, using a power of 0.8 and a two-sided alpha of 0.05 for our primary analysis that will compare pre and post tributyrin, we arrive at a total sample size of 20.

Pitfalls, problems and alternative strategies

Ineligibility and attrition rate:

Protection of Human Subjects

i) Human Subjects

Description of subject populations:

i) Healthy control persons, M/F, age 45 years and over without a history of neurological or psychiatric disease or significant GI disease or uncontrolled medical comorbidity will be recruited (net n=4).

ii) PD (net n=16) Diagnosis of PD in PD participants (M/F, age 45 years and over) will based on the United Kingdom Parkinson's Disease Society Brain Bank Diagnostic Research Criteria (patients with clinically significant dementia will be excluded (i.e., fails informed assent screening criteria and has significant evidence of iADL functions, such as ability to take own medications while administering the pre-consent screening questionnaire).

Exclusion criteria:

1. *Exclusion*: (a) subjects with contra-indications to MR imaging, including pacemakers or severe claustrophobia (for participants undergoing imaging); (b) Evidence of large vessel stroke or mass lesion on MRI; (c) Regular use of anti-cholinergic, benzodiazepines or neuroleptic drugs, (d) history of significant GI disease, (e) significant metabolic or uncontrolled medical cormorbidity, (f) poorly controlled diabetes, (g) pregnancy or breast feeding, (h) dementia requiring informed assent, (i) suicidal ideation.

Recruitment: Subjects will be recruited from the University of Michigan Movement Disorder clinics, UM research studies website, and/or advertisement/flyers.

ii) Sources of Materials:

Information gathered specifically for this research project includes test results from clinical test scales, psychometric cognitive measures, neurobehavioral rating scales, clinical motor data, questionnaires, and data derived from the butyrate and FDG PET and from brain MRI.

iii) Potential Risks:

Confidentiality of Research Information: The research data to be collected from subjects will consist of confidential information relating to clinical, motor, cognitive, neurobehavioral and neuro-imaging functions. These research data are not intended for entry into the subjects' clinical medical records. However, the data remain potentially discoverable. This may lead to violation of privacy and embarrassment of the subject.

Clinical Testing: Risks in regard to the clinical assessment are limited to fatigue, frustration, discomfort, and momentary embarrassment that may occur when one experiences difficulty performing a task or learning a new skill.

Risks associated with clinical, neuropsychological and behavioral testing: This study involves cognitive tests and questionnaires. The cognitive tests are not harmful but some people find them frustrating and concerning. Risks in regard to the neuropsychological and behavioral assessment are limited to fatigue, frustration and momentary embarrassment that may occur when one experiences difficulty performing a task or learning a new skill. Some people may be uncomfortable/embarrassed disclosing personal information or become a little nervous about memory testing, and may experience discomfort or become tired. Study team members are very well experienced in the assessment of older individuals and persons with dementia, understanding the need for breaks, gentle reassurance, or reinforcement. All assessments will be stopped if requested by the participant.

MRI scan: There is a substantial risk to persons who have metallic objects inside their bodies, as the magnet in the MR scanner can cause these to move. Consequently, participants with pacemakers or metallic objects located in the eye, ear, brain or blood vessel walls will be excluded. Participants who developed claustrophobic anxiety during scanning found that this fear dissipated within 15 min while remaining in the scanner, or as necessary, after exiting the scanner. There also is the potential that imaging could reveal a previously unrecognized but pre-existing abnormality. Many such abnormalities are not clinically significant, but they may cause anxiety or require further investigation by a personal physician. If one of the investigators identifies such an abnormality, they will contact the personal physician, who will arrange for appropriate care.

PET-CT scans & venipuncture: Insertion of a catheter for intravenous injection of the PET radiopharmaceutical may be commonly associated with slight pain or bruising at the puncture site and rare chance of infection. Participation in this research study will involve low level exposure to radiation associated with the PET, CT and DEXA scans.

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.

During the course of this study, the participant will be exposed to radiation from the two [^{11}C]butyrate PET, the two [^{18}F]FDG PET-CT and two DEXA scans. The risks associated with the amount of radiation exposure participants receive in this study are considered very rare and comparable to every day radiation exposure risks.

The biological effect of radiation in humans is measured in terms of Sieverts (Sv) or mSv (1/1000 Sv), which is a unit of uniform whole body exposure. The maximum radiation exposure (assuming a person will complete all 4 PET scans) a participant will receive from the CT, [^{11}C]butyrate PET and [^{18}F]FDG PET scans is equivalent to a uniform whole body dose of 21.2 mSv, which is approximately 42.4% of the annual radiation exposure (50 mSv) permitted to radiation workers by federal regulations. The participant will be instructed to use the bathroom and urinate as soon as possible

after the PET scans to minimize bladder exposure. The radiation the participant will be exposed to from each DXA scan is 0.0310 mSv, which is significantly less than 1% of the permitted annual radiation exposure.

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 you 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, and urine sampling may be performed on subjects at baseline and post-interventions as an additional screening measure.

Risks associated with tributryin: The risks to participants are very minimal. Although tributyrin is taken for symptoms of irritable or inflammatory bowel disease it is possible that nausea or indigestion may occur. However, these gastrointestinal side effects are both rare and mild. Tributyrin is received GRAS (Generally Regarded As Safe) certification from the FDA, is commercially available in the United States and prior work has shown that the dosing regimen proposed in this study has been found to be safe and generally well-tolerated.

Data Study Monitoring Plan (DSMP): Drs. Bohnen and Albin will act as the Data Safety Monitoring Plan members for this study. All adverse events and other study related events will be discussed at monthly investigator meetings. Study team will document any adverse events specifically related to study participation (imaging, clinical visit, at-home supplement and/or device usage, etc.). 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 within 24 hours.
For risk of suicidality (if participants select answer responses 2 or 3 for question 9 on the Beck Depression Inventor, i.e. 'I would like to kill myself' (answer 2) or 'I would like to kill myself if I had a chance' (answer 3) then the staff will hand out the UM Depression brochure, refer to the contact information and also inform Dr. Bohnen or Dr. Albin who will discuss this with the participant. The face to face conversation with the physicians must occur during that visit and must not be deferred. There must be a safety plan in place before the patient can leave the building.

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