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PROTOCOL Amendment 1

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Investigational product and support:
Ultragenyx

Investigator signature page

I agree to implement and conduct this study diligently and in strict compliance with the protocol, good clinical practices and all applicable laws and regulations.

I have read this protocol in its entirety and I agree to all aspects.

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Investigator Printed Name	**Signature**	**Date**

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Investigator Printed Name	**Signature**	**Date**

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1. DEFINITIONS AND ABBREVIATIONS

Alzheimer's disease: A clinical syndrome involving a memory disorder and impairment in at least one other cognitive domain, both of which significantly interfere with social function or activities of daily living.

Anaplerosis: The process by which citric acid cycle intermediates are replaced by molecules such as pyruvate or propionate. Insufficient anaplerosis may contribute to increasing the risk of brain energy exhaustion, a problem that can potentially be avoided or diminished with *triheptanoin (see Triheptanoin below)*.

CMR-A (cerebral metabolic rate of acetoacetate): The brain uptake of the ketone, acetoacetate, as determined by PET using ^{11}C-acetoactate and expressed as μmol/100 g/min.

CMR-G (cerebral metabolic rate of glucose): The brain uptake of glucose as determined by PET with the tracer, ^{18}F-flurodeoxyglucose (FDG), and expressed as μmol/100 g/min.

PET (positron emission tomography): An imaging method we use to quantify brain uptake of ketones (^{11}C-acetoacetate) and glucose (FDG). In our PET protocol, the two tracers are injected sequentially on the same afternoon so as to necessitate only one visit and so as to minimize biological variability between scans obtained on different days.

Diffusion Magnetic Resonance Imaging: MRI modality involving the reconstruction of white matter fiber bundles (tracts) and measures of white matter integrity.

FDG (^{18}F-flurodeoxyglucose*):* The PET tracer for tissue glucose uptake studies.

Investigational product: Triheptanoin (UX-007; Ultragenyx, Novato, CA, USA)

Ketones: Mainly *β-hydroxybutyrate* and *acetoacetate*, the four-carbon *'ketone bodies'* which are the key alternative brain fuels to glucose. β-Hydroxybutyrate is converted to acetoacetate and then to acetyl CoA which enters the citric acid cycle. Our PET ketone tracer is carbon-11 (^{11}C)-acetoacetate. Molecules that give rise to ketones are *ketogenic*, i.e. *triheptanoin* (see below).

Mild cognitive impairment: Combination of (i) informant-corroborated memory complaint, (ii) mild memory deficit on objective cognitive testing, (iii) normal general cognitive function, (iv) normal activities of daily living, and (v) absence of Alzheimer's disease or dementia.

Neuroimaging portfolio: Our neuroimaging portfolio combines dual tracer PET (^{11}C-acetoacetate and FDG) with diffusion MRI, resting state functional MRI and volumetric MRI.

REC (Research Ethics Committee of the Research Center on Aging). The committee authorized to approve all human research at our institution, and to which all SAEs will be reported.

Resting state functional magnetic resonance imaging: MRI modality that shows the areas of the brain that are synchronized when the subject is at rest, i.e. when <u>not</u> performing a task.

SAE (Serious adverse event): An SAE is any untoward medical occurrence that, at any dose of the investigational product, results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalisation, results in disability or incapacity, is a congenital abnormality or birth defect, or is a significant medical event.

Triheptanoin: An edible medium chain triglyceride oil composed of three heptanoic acids (seven carbon saturated fatty acids). The potential importance of triheptanoin for brain energy metabolism during aging is that it is both ketogenic and anaplerotic. Unlike classical eight- or ten-carbon medium chain triglycerides, triheptanoin gives rise to both four and five carbon ketones.

Volumetric MRI: A term referring to the MRI modality that measures cortical thickness, and volume of regional grey matter, white matter, ventricles and cerebrospinal fluid surrounding the brain.

2. BACKGROUND

Overview

Glucose uptake and utilization is significantly lower in the brain of patients with Alzheimer's disease, a situation that progressively leads to brain energy exhaustion. Without sufficient energy for brain cells, normal communication between neurons is impaired leading to impaired memory and cognitive function. We believe that there is good evidence that brain energy exhaustion not only underlies and contributes to the development of Alzheimer's disease but could be at least partially correctable by *triheptanoin*.

The novelty of this trial is fourfold: (i) Its rationale is that the core issue leading to Alzheimer's disease is a metabolic (energy) problem with glucose in the brain. Although well-supported by the literature, this is still very much an emerging perspective. (ii) Replacing deteriorating brain glucose uptake by a dietary supplement that raises ketones has been tested before but has not been convincingly shown to improve cognition in Alzheimer's disease. We are proposing to use triheptanoin which should be more effective than classic ketogenic supplements but has not been tested in humans to delay declining brain glucose metabolism in older people. (iii) How increased availability of ketones affects human brain energy metabolism, structure and function is unknown. Our cutting-edge neuroimaging portfolio provides us with a unique capability to examine the effect of triheptanoin on brain energy metabolism (glucose and ketones), structure of white matter tracts, and functional connectivity across brain regions. Our neuroimaging portfolio is unique because it includes a measure of brain ketone uptake, a key capability for this study which only our group has at the moment. (iv) We will conduct this study in older people with lower brain glucose uptake. Nothing has been done to establish how brain energy metabolism, functional connectivity and/or white matter structure may be changing in older people in ways that predispose to Alzheimer's disease, so this is novel.

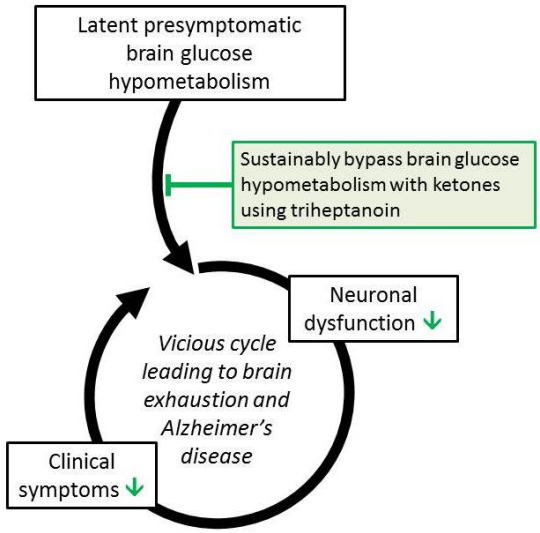
Brain exhaustion in Alzheimer's disease - cause, consequence or both?

Brain function requires a lot of energy, i.e. ~20% of the body's energy intake goes to the brain despite it weighing only ~2% of body weight in adults. For >30 years now, positron emission tomography (PET) studies in Alzheimer's disease have shown that uptake of glucose, the brain's main fuel, is reduced in certain brain regions by 30-40% (reviewed by Silverman et al. 2001; Cunnane et al. 2011). The brain glucose uptake deficit is predominantly in the parietal cortex, temporal cortex, precuneus and posterior cingulate. This specific regional pattern of brain glucose hypometabolism helps distinguish Alzheimer's disease from normal aging and other forms of dementia (Silverman et al. 2001). With the wide interest in beta-amyloid and plaque accumulation, the general interpretation of these studies was and continues to be that brain glucose uptake decreases in Alzheimer's disease due to synaptic deterioration and neuronal dysfunction: thus, as a consequence of needing less glucose, brain glucose uptake declines.

While it is certainly true that brain glucose uptake will decline as a consequence of declining neuronal (especially synaptic) function, there are several clear examples of conditions in which lower brain glucose uptake is present pre-symptomatically in those at risk of Alzheimer's disease, i.e. before the emergence of any clinically measurable cognitive deficit associated with Alzheimer's disease. These examples include – (i) Presenilin-1 carriers, who are essentially guaranteed to get early onset Alzheimer's disease (Scholl et al. 2011), (ii) Apolipoprotein E4 carriers, who as heterozygotes are at

four fold and as homozygotes are at 19 fold higher risk of Alzheimer's disease (Reiman et al. 2004), (iii) Maternal familial history of Alzheimer's disease (Mosconi et al. 2010), and (iv) Type 2 pre-diabetics and/or insulin resistance, which are the most important lifestyle risk factors for Alzheimer's disease (Baker et al. 2011; Cunnane et al., unpublished). In the Presenilin-1 carriers, apolipoprotein E4 carriers, and in those with a maternal family history of Alzheimer's disease, the lower regional brain glucose uptake is present 20-30 years before the expected decline in cognition; in our young women with mild insulin resistance, the lower brain glucose uptake is present at 24 years old, i.e. 40 years before the typical onset of Alzheimer's disease (Cunnane et al., unpublished).

Thus, low brain glucose uptake in moderately advanced Alzheimer's disease is logically in part a product of less demand for fuel caused by neuronal dysfunction. However, before the clinical onset of Alzheimer's disease symptoms, the examples given above show that latent pre-symptomatic brain glucose hypometabolism potentially also contributes towards cognitive decline (Veech et al. 2004; Henderson et al. 2009; Cunnane et al. 2011; Mamelak 2012; Castellano et al. 2015). We have proposed that latent glucose hypometabolism gradually leads towards brain energy exhaustion, which could in turn lead to neuronal dysfunction, gradual cognitive decline and further glucose hypometabolism creating a vicious cycle leading to dementia (Cunnane et al. 2011; Castellano et al. 2015; see Figure on the right).



Brain energy exhaustion in Alzheimer's disease is specific to glucose

When glucose supply to the brain is low, *ketones* (or *ketone bodies - beta-hydroxybutyrate and acetoacetate*) are the only fuels that replace glucose for the brain in a quantitatively important sense. This makes the brain unique relative to other organs all of which routinely use fatty acids to replace glucose on a daily basis, ex. between meals or during fasting or sleep (Owen et al. 1967; Hasselbalch et al. 1996; Maalouf et al. 2009; Cunnane et al. 2011). Ketones access the brain via monocarboxylic acid transporters and enter the citric acid cycle at acetyl-CoA, so they generate ATP in the brain independently of both glucose uptake and glycolysis.

The brain has two strategies to acquire its main fuels – a 'pull' strategy for glucose and a 'push' strategy for ketones. The 'pull' strategy for glucose refers to the increase in brain glucose uptake that is driven by brain cells in response to their activation, i.e. once activated, neurons 'pull in' glucose from the blood to replace ATP. The 'push' strategy for ketones refers to the increase in brain ketone uptake that is driven by increased plasma ketones which rise when plasma glucose and insulin decrease, i.e. higher blood ketones 'push' into the brain when blood glucose is declining. The push strategy for ketones is a critical component of our novel solution to delaying Alzheimer's disease by preventing brain energy exhaustion.

The main PET tracer for brain fuel uptake studies is the glucose analog - ^{18}F-flurodeoxyglucose [FDG]). Until we developed carbon-11-acetoacetate (^{11}C-AcAc) to use as a PET ketone tracer (Nugent et al. 2014a; Castellano et al. 2015), there was no way to measure brain ketone uptake on a region-

by-region basis in humans. Being able to compare the brain uptake of glucose and ketones is critical to understanding both the nature of the early stage of brain energy exhaustion in older people and possible ways to correct or delay it.

Using our dual tracer PET protocol (injecting ^{11}C-AcAc followed by FDG all within 3 hours on the same day), we have recently reported that, as expected, regional brain glucose uptake was about 17% lower in mild Alzheimer's disease. However, brain ketone uptake was not different compared to cognitively normal, age-matched controls (Castellano et al. 2015). Our PET results confirm two older studies done using arterio-venous difference across the whole brain to measure glucose and ketone uptake in Alzheimer's disease (Lying-Tunell et al. 1981; Ogawa et al. 1996). Collectively, these three studies have important implications for developing a strategy to prevent brain energy exhaustion in older people because they suggest that brain ketone uptake is still normal even though brain glucose uptake is regionally and globally decreased in mild-moderate Alzheimer's disease. At least early in Alzheimer's disease, it is therefore incorrect to refer to 'brain hypometabolism' as a general phenomenon because it does not apply to ketones and appears to be specific to glucose.

Ketones, ketogenic diets and ketogenic supplements

Small clinical trials show that mildly raising blood ketones improves cognitive outcomes in *mild cognitive impairment* or mild-moderate Alzheimer's disease. This is typically done using a ketogenic diet (Krikorian et al. 2012) or a ketogenic supplement providing eight and/or ten carbon 'classical' *medium chain fatty acids* (Reger et al. 2004; Henderson et al. 2009). The fact that brain ketone uptake is still normal in mild-moderate Alzheimer's disease (Lying-Tunell et al. 1981; Ogawa et al. 1996; Castellano et al. 2015) suggests that the mechanism by which ketones improve cognitive outcomes in mild cognitive impairment or Alzheimer's disease is at least in part by providing the brain with an alternative fuel that bypasses the regional brain glucose hypometabolism. This is the physiological means by which the brain is fueled during fasting because ketone transport into the brain and their access to the citric acid cycle is independent of glucose or the glycolytic pathway. Therefore, with ketones as an alternative brain fuel to glucose, the risk of further neuronal dysfunction due to progressive brain exhaustion would appear to be reduced.

As a result, in principle, cognition could be stabilized or possibly even improved. Ketogenic diets and supplements have been used clinically to treat intractable epilepsy for decades (Couch et al. 1999; Neal et al. 2008) and are being explored as treatments in Alzheimer's disease (Henderson et al. 2009) and brain cancer (Seyfreid et al. 2014). Ketogenic supplements may be beneficial to counteract the symptoms of hypoglycemia in Type 1 diabetics being aggressively treated with insulin (Page et al. 2009). Some ketogenic supplements are already on the market (Axona®, Betaquik®, Fuel for Thought®) and others are undergoing clinical trials.

To achieve mild nutritional ketosis requires one of the following two strategies: (i) Produce ketones from long-chain fatty acids which are 16-18 carbon fatty acids that make up the common dietary fats and body fat stores. This strategy requires sustained hypoinsulinemia achieved only by severely limiting carbohydrate intake to <5% of total energy intake and increasing fat intake to 80-90% of energy. Such a very high fat 'ketogenic diet' permits the body to release more long chain fatty acids from fat stores and from dietary fat in order to drive ketone production by the liver. However, the major constraint is that insulin production must remain low, i.e. carbohydrate intake must be very low. (ii) Produce ketones from medium-chain fatty acids (8-12 carbon fatty acids), which involves taking a *medium chain triglyceride* supplement. The big advantage is that this approach avoids the dramatic change in macronutrient intake required by a ketogenic diet. Medium chain fatty acids

bypass the carnitine-dependent step in fatty acid transport for mitochondrial beta-oxidation and are moderately ketogenic. Both strategies safely raise blood ketones but also have their disadvantages: ketogenic diets are hard to maintain in adults because the slightest increase in carbohydrate intake breaks the ketosis; ketogenic supplements are simpler but have gastrointestinal side-effects.

Ketogenesis plus anaplerosis – a better way to sustain brain energy supply

One reason that classic ketogenic diets or supplements have only a marginal to modest beneficial effect on cognition in older people may be because long-term use of ketogenic diets or ketogenic supplements based on even-numbered medium chain fatty acids appears to deplete citric acid (Krebs') cycle intermediates on the way towards producing energy, i.e. ATP (Mochel et al. 2005; Brunengraber and Roe 2006; Deng et al. 2009). Molecules in the citric acid cycle such as oxaloacetate are intermediates contributing to the cyclical replacement of citric acid but must themselves be replaced for the cycle to continue efficiently generating ATP. Replacing the citric acid cycle intermediates is a process called *anaplerosis*. The citric acid cycle is important for energy production but it is also needed to make several molecules, including the neurotransmitter, gamma-aminobutyric acid.

When glucose is the main fuel for the brain, it supplies both the carbon to make the ATP and the carbon for the intermediates of the citric acid cycle. As an analogy, glucose acts like both the gasoline and the motor oil for the engine. However, unlike glucose, the even-carbon numbered ketones produced from most ketogenic supplements do not replace citric acid cycle intermediates, i.e. ketones provide the gasoline but not the motor oil. This is because virtually all medium- or long-chain fatty acids in the diet are 'even numbered' i.e. 8, 10, 12, 14, 16, 18, 20 carbons in length, so do not get converted to oxaloacetate.

The process by which carbon to make citric acid cycle intermediates is anaplerosis, while the opposite process (the depletion of the intermediates) is *cataplerosis* (Brunengraber & Roe, 2006). Ideally, there is a balance between anaplerosis and cataplerosis but long-term ketogenesis tends to be cataplerotic, which curtails the efficacy of both ketone oxidation as an energy substrate and the production of neurotransmitters such as gamma-aminobutyric acid which may be part of the beneficial effect of ketogenesis (Yudkoff et al. 2005; Roy et al. 2015).

Neuroimaging portfolio: linking brain fuel metabolism, structure and functional connectivity

Our neuroimaging portfolio integrates dual tracer PET with three magnetic resonance imaging (MRI) modalities. We developed this portfolio with the specific goal of measuring how brain metabolic vulnerability develops in older people and to find out how metabolic deterioration in the brain affects white matter integrity and functional connectivity. Our neuroimaging portfolio consists of four modalities:

(i) Dual tracer brain PET. Our dual tracer brain PET protocol with FDG and ^{11}C-AcAc provides an important and unique (at present) window on brain energy metabolism because we quantify uptake of both the brain's main fuels. Our quantification involves measuring the actual metabolic uptake of FDG and ^{11}C-AcAc and expressing these data as the *cerebral metabolic rate of glucose (CMR-G)* and *^{11}C-AcAc (CMR-A),* respectively (Nugent et al. 2014a, 2014b; Castellano et al. 2015). The units for CMR-G and CMR-A are *μmol/100 grams/minute*, which combine the rate constants for FDG and ^{11}C-AcAc uptake with their plasma concentrations. We obtain CMR-G and CMR-A values for the whole brain and for multiple brain regions. These values are the only way to know the <u>actual deficit</u> in

glucose uptake in a specific brain region and, hence, the therapeutic target for the triheptanoin in the present study. The more commonly used statistical parametric mapping procedures greatly simplify the estimation of the glucose deficit and mapping it onto the brain. However, they only show a statistical difference between brain regions and do not provide a true measure of the quantitative difference between regions. Hence, we know the actual deficit in brain glucose uptake and can calculate both the therapeutic target and the quantitative effect of triheptanoin on brain fuel uptake.

Until we developed ketone PET, there was no marker of regional brain ketone metabolism and no explanation of how ketogenic diets or supplements would potentially improve cognition. This new window on brain fuel metabolism is important because, in rats, our dual tracer brain PET method shows the expected increase in brain ketone uptake in response to mild nutritional ketosis, but also shows an unexpected increase in brain glucose uptake (Pifferi et al. 2011; Roy et al. 2012). We are in the process of confirming this observation in humans on a short-term ketogenic diet (Courchesne-Loyer et al 2015; see Preliminary Results). Indeed, it would be very interesting if any treatment could not only increase brain ketone uptake but also delay or reverse the gradual decline in brain glucose uptake.

(ii) Volumetric MRI. Volumetric MRI (vMRI) refers to the T1-MRI-based measurement of cortical thickness and regional gray matter, white matter and cerebrospinal fluid volumes by MRI. We compute these volumes for multiple brain regions. Like others, we have shown cortical gray matter and sub-cortical structures (hippocampus, thalamus) are 15-25% smaller in early Alzheimer's disease (Nugent et al 2014a, 2014b; Castellano et al 2015). These measurements are in themselves important indicators of change associated with aging and risk of cognitive decline. In addition, they are essential to correctly measure CMR-G and CMR-A.

(iii) Diffusion and rsfMRI. We use state-of-the-art fiber tractography that can identify fiber crossings (Descoteaux et al. 2009; Girard-Tremblay et al. 2014) and automatic *tractography dissection* to reconstruct the brain's white matter fiber bundles or *tracts*. dMRI tractography is therefore a powerful tool to dissect the brain's white matter into its specific structural regions of interest. As a result, the integrity of white matter can be assessed both globally and for specific tracts connecting regions of the brain that are known to be highly synchronized, such as the default mode network which is measured using rsfMRI. Using our PET data, particularly with FDG, we are using the grey matter and sub-cortical regions with low FDG uptake in Alzheimer's disease to study the integrity of white matter bundles inter-connecting these regions to establish whether low FDG uptake is associated with deteriorating white matter bundles or whether the former is independent of the latter. White matter bundles connecting to the thalamus are of particular interest in this respect because the thalamus shows low FDG uptake in Alzheimer's disease (Castellano et al 2015).

(iv) Dual tracer PET, rsfMRI and neuropathology. The brain's 'default mode network' as measured by *resting state functional magnetic resonance imaging* (rsfMRI) correlates well with the pattern of brain amyloid-beta accumulation in Alzheimer's disease (Sperling et al 2009; Vlassenko et al. 2010) and glucose preference (Nugent et al. 2014a). Thus, regions of the brain that prefer glucose over ketones appear to be the same regions that remain functionally connected or synchronized when the brain is at rest, i.e. when a specific task is NOT being performed. Remarkably, these brain regions are the same ones that tend accumulate beta-amyloid later in life. This suggests that the vulnerability of certain brain regions to neuropathology later in life is linked in some way to metabolic fuel utilisation independently of age, even in young adults (Nugent et al. 2014a; Nugent et al. 2015).

Rationale

If ketogenic supplements are to be exploited to prevent or delay brain fuel exhaustion, then establishing the long-term sustainability of ketogenesis becomes a critical therapeutic goal. Hence, a substance that is both ketogenic and anaplerotic and appropriate for human consumption is needed. Such a ketogenic-anaplerotic substance appears to exist in the form of triheptanoin which is a medium chain triglyceride composed of three seven carbon *heptanoic acids* on a glycerol backbone. Triheptanoin is more effective than its even-numbered homologue, trioctanoin, in animal models of both epilepsy (Borges and Sonnewald 2012; McDonald et al. 2014) and Alzheimer's disease (Aso et al. 2013).

Oral daily triheptanoin has been shown to be safe and well-tolerated in children for months at a time (Pascual et al. 2014) as well as in adults (Roe et al. 2009). It is also more ketogenic than the more common 8 and 10 carbon even-chain medium chain triglycerides in children with pyruvate carboxylase deficiency (Mochel et al. 2005). Prior experience with classical even chain medium chain triglycerides and with triheptanoin shows that the dose should be up to 1 g/kg/day taken with meals. Medium chain triglycerides resolve some of the symptoms but are not a cure for diseases such as glucose transporter deficiency (Pascual et al. 2014), so the treatment regimen needs to be effective and well-tolerated over the long-term. As with the classical even-chain medium chain triglycerides, the adverse effects of triheptanoin are mostly gastrointestinal distress, and can usually be resolved by splitting up the daily dose, taking it with meals, gradual dose titration and efficient mixing with other foods.

Hence, the principle that ketogenesis with anaplerosis would be more effective than ketogenesis alone is at least provisionally supported by a small but growing literature on the safety and efficacy of triheptanoin in clinical and pre-clinical research. To our knowledge, no one has yet reported using triheptanoin in humans with Alzheimer's disease or mild cognitive impairment, so its application in these conditions is scientifically unproven but holds considerable potential. Hence, the BEAT7 Study will be a proof-of-principle for a new use of triheptanoin, i.e. a Phase 1b study aiming to delay the risk of cognitive decline in older people by bypassing or maybe correcting lower brain glucose uptake. In older people, brain glucose hypometabolism is particularly prevalent in the frontal cortex and is present before the onset of measurable cognitive decline (Nugent et al. 2015).

3. PRELIMINARY DATA

Ketogenic diet and dual tracer brain PET

We now have a database with n>100 dual tracer (FDG and ^{11}C-AcAc) PET scans in cognitively-healthy younger and older people, some of which has been reported (Nugent et al. 2014a, 2014b; Castellano et al. 2015). Our CMR-G and CMR-A values for the brain as a whole, for grey and white matter and for specific brain regions in healthy controls are within expected limits so this is a robust, safe and well-validated protocol. The present study will be our first to use neuroimaging before and after triheptanoin. However, we have assessed brain fuel uptake in adults before and four days after a ketogenic diet and have shown that this treatment increases brain ketone uptake by ~8 fold (Courchesne-Loyer et al. 2015). These preliminary results clearly show that brain ketone uptake increases rapidly and markedly when ketone production is strongly but safely stimulated by a ketogenic diet. The advantage of our PET method is that we can quantify and compare ketone (CMR-A) and glucose (CMR-G) uptake across multiple brain regions, which is essential to determining whether triheptanoin alters the regional glucose preference seen across the brain (Nugent et al.

2014a; Vlassenko et al. 2010). Our proposed dose of triheptanoin (1 g/kg body weight/day) is within the therapeutic target to bypass the 10% brain glucose uptake deficit seen in older people (Cunnane 2011; Courchesne-Loyer et al 2013; Nugent et al. 2014a; 2014b; Nugent et al. 2015). Hence, we will be able to determine whether improved brain fuel uptake actually occurs in the regions experiencing glucose hypometabolism in older people.

4. STUDY OBJECTIVE

To use a multi-modal brain imaging portfolio to quantitatively assess whether brain energy metabolism (glucose and ketones), structure or functional connectivity change in older people with frontal glucose hypometabolism after 28 days on an oral dose of 1 g/kg/day of triheptanoin.

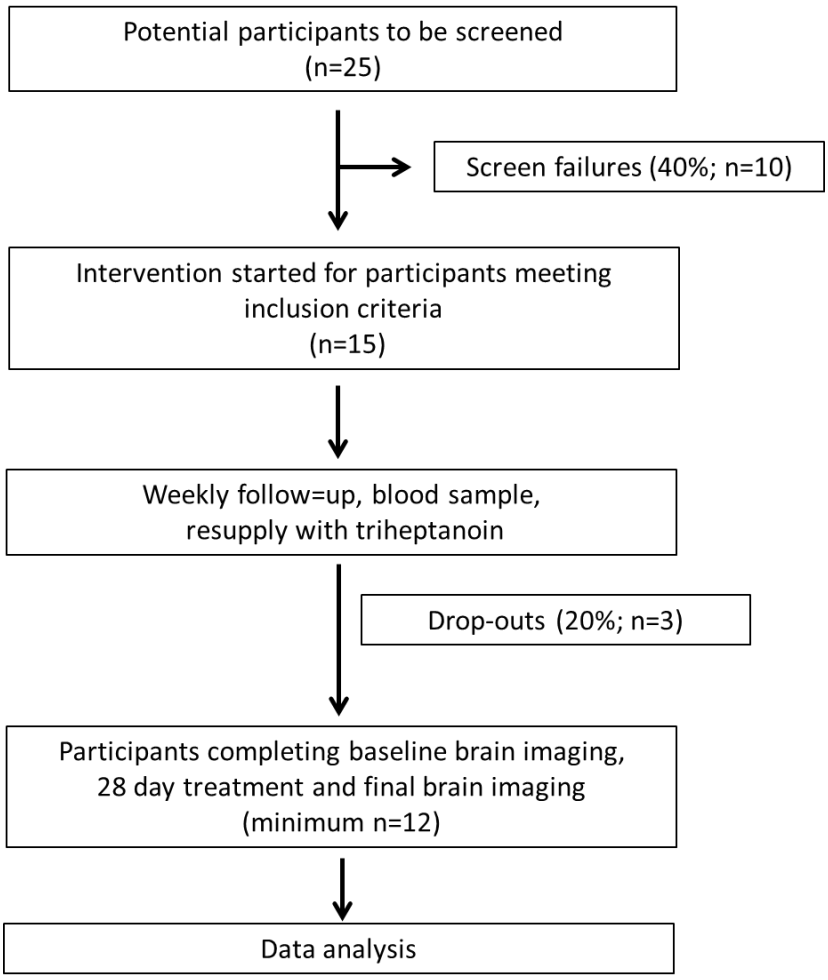
5. STUDY HYPOTHESIS

Our multi-modal brain imaging portfolio provides a unique opportunity to quantitatively assess whether brain energy metabolism (glucose and ketones), structure or functional connectivity change in older people with frontal glucose hypometabolism after 28 days on an oral dose of 1 g/kg/day of triheptanoin.

6. STUDY DESIGN

Overview

It will be a Phase 1b study assessing a new purpose for triheptanoin in an older population. The intervention will last 28 days. The co-primary endpoints will be the quantitative change in brain ketone and glucose uptake measured using positron emission tomography.



7. SELECTION AND WITHDRAWL OF PARTICIPANTS

7a. Study population

The target population will be people ≥65 years old with frontal glucose hypometabolism as determined by FDG-PET. They will have no objective evidence of mild cognitive impairment or Alzheimer's disease at screening.

7b. Selection criteria

Inclusion criteria:

- Men and women ≥65 years old;
- Score ≥26/30 on the Montreal Cognitive Assessment;
- ≥10% lower brain glucose uptake in the frontal cortex as determined by PET imaging (Nugent et al. 2015).

Exclusion criteria:

- Score <26/30 on the Montreal Cognitive Assessment;
- Medications likely to affect the primary cognitive outcome;
- Medical or psychiatric conditions that could interfere with study participation (Peterson et al. 2005);
- Fasting plasma glucose ≥7.0 mM (to avoid recruiting diabetics or pre-diabetics, both of which are risk factors for cognitive impairment in older persons (Mortimer et al. 2010) and also inhibit ketogenesis (Fukao et al. 2004);
- Clinically-significant gastro-intestinal disease/conditions;
- Clinically-significant liver disease/dysfunction : ALT ≥37 UI/L, AST ≥36 UI/L, Total bilirubin ≥26 µmol/L;
- Clinically-significant renal disease/dysfunction : creatinine ≥92 µmol/L, glomerular filtration rate <60 ml/min/1.73 m^2 or >90 ml/min/1.73 m^2;
- Clinically-significant cardiac disease/conditions;
- Clinically-significant abnormal coagulation laboratory results or coagulation disorders at screening;
- Poorly controlled dyslipidemia (total cholesterol ≥6.2 mmol/L or triglycerides ≥2.20 mmol/L)
- Hypertension: ≥140/90 mmHg;
- Substance abuse;
- Already on MCT supplementation;
- Visual or hearing impairment impeding comprehension;
- Non-French speaking;
- Any condition with life expectancy less than 5 years;
- Institutionalized or intending to move out of area within 1 year;
- Participation in other intervention trials.

7c. Participant recruitment and, screening

Recruitment: Prospective participants will be recruited from our bank of over 50 older persons in whom FDG-PET has already been assessed. If necessary, participants will also be recruited using publicity in public spaces, radio, newspapers, bulletins and social media in the Sherbrooke area. Since prospective participants will lead essentially normal lives, memory clinics are not likely to be useful for recruitment. For individuals not wishing to participate in the study after screening, or who meet one or more of the exclusion criteria, the research nurse will only record age, gender, and reason for study exclusion.

Screening: The screening will consist of a – (i) medical history, (ii) cognitive assessment to exclude those with prodromal or overt Alzheimer's disease, and (iv) an FDG PET scan. Identification of individuals with mild cognitive impairment or Alzheimer's disease will be based on the score on the Montreal Cognitive Assessment. Those scoring <26/30 on the Montreal Cognitive Assessment will be excluded.

We have experience with routine cognitive testing to screen for declining memory or executive function associated with mild cognitive impairment in older people (Nugent et al, 2014a; Nugent et al, 2014b, Castellano et al. 2015). The BEAT7 Study lead clinical investigator, Dr. Tamas Fulop, will confirm the appropriateness of each person invited to participate. We have planned for a 40% screen failure rate (Yurko-Mauro et al. 2010).

Control group: As a Phase 1b study in which the primary outcomes are based on imaging outcomes obtained pre- and post-intervention, no placebo or control group will be include in the project. Variation of the different outcome measures are unlikely to change significantly during one month without an intervention, as suggested by test-retest which show not significant difference after a 6 month period. (Schaefer et al. 2000).

7d. Intervention

Triheptanoin group: The target daily dose of triheptanoin will be 1 g/kg/d, or approximately 70 g/d. It will be divided into four daily doses, one of which will be consumed at each meal and one with an evening snack. The triheptanoin is to be mixed with or blended into a variety of cold foods or drinks that have been shown to be effective for this purpose: plain or fat-free yogurt, whole grain hot cereal, fat-free cottage cheese, fat-free milk, fat-free low carb pudding, smoothie, applesauce or baby food.

To improve tolerance and reduce the risk of side-effects, the dose of triheptanoin will be titrated upwards according to a previously tested schedule starting at 0.25 g/kg/day (about 4 g/meal) during days 1-7, increasing to 0.5 g/kg/day during days 8-14 (about 9 g/meal), increasing to 0.75 g/kg/day during days 15-21 (about 13 g/meal), and finally to 1.0 g/kg/day during days 22-28 (about 17 g/meal).

Retention: Retention in the study will be encouraged by weekly meetings with the research nurse to collect the triheptanoin supplement and provide a blood sample for a measure of compliance.

8. STUDY DURATION

The triheptanoin will be given for 28±2 days. The two day leeway either way is to allow for last-minute difficulties that can arise in scheduling the brain imaging. Brain ketone uptake increases within days of consuming a ketogenic diet (Courchesne-Loyer et al, 2015). However, the main effect of increased brain ketone uptake may be on a brain structural or functional parameter that takes longer to change than brain fuel uptake, so the study will last 28 days to try to capture effects on parameters measured using our neuroimaging portfolio.

9. RESPONSE MEASURES

9a. Co-primary outcome measures

The co-primary outcomes will be change in <u>global</u> glucose uptake (CMR-G using FDG) and change in <u>global</u> ketone uptake (CMR-A using ^{11}C-AcAc) uptake in grey matter after 28 days on the triheptanoin treatment compared to pre-treatment values.

9b. Secondary outcome measures

The secondary outcome measures will be change in post-treatment values versus pre-treatment values for:

(i) CMR-A in the frontal cortex.
(ii) CMR-G in the frontal cortex.
(iii) MRI-based parameters: brain connectivity by rsfMRI, integrity of brain white matter tracts by dMRI, and regional brain volumes by vMRI.
(iv) Cognitive score using the Montreal Cognitive Assessment.

10. STUDY METHODS AND PROCEDURES

Events schedule

Visit day		**0**	**Day 7, 14, 21**	**28**
Visit name	**Screening**	**Baseline**		**Completion**
Written informed consent	X			
Demographics	X			
Medical history	X			
Montreal Cognitive Assessment	X			X
Blood sample for lab assessments[1] and vital signs (blood pressure, pulse)	X	X	X	X
FDG-PET[2]	X			
Neuroimaging[3]		X		X
Adverse events report[4]	X	X	X	X
Dispense supplement		X	X	

[1] Plasma beta-hydroxybutyrate, acetoacetate, acylcarnitine profile, glucose, free fatty acids, triglycerides, total cholesterol, lactate, complete blood count, electrolytes, pH, creatinine, and liver enzymes.

[2] Needed during screening unless participant already has an FDG-PET scan on file within the past 6 months.

[3] FDG and ketone PET; volumetric, functional and diffusion MRI

[4] All serious adverse events will be reported if and when they arise

Written informed consent

All prospective participants will provide written, informed consent prior to enrollment.

Demographics, and medical history

During screening a medical history including vital signs and anthropometry will be obtained by the research nurse associated with the BEAT7 Study.

Neurological assessment

Cognitive performance will be assessed at screening and at the end of the 28 day treatment period by the Montreal Cognitive Assessment.

Laboratory assessments

Blood samples will be collected for pre-screening and to measure compliance (See Events schedule). Cunnane's lab does these analyses routinely (Nugent et al. 2014a; Castellano et al. 2015).

Neuroimaging

All prospective participants will undergo an FDG PET scan prior to enrollment; they will not be enrolled unless they have ≥10% lower brain glucose uptake in the frontal cortex.

The PET component of our neuroimaging portfolio is essential for quantifying three aspects of brain fuel uptake in this study - (i) pre-intervention brain glucose uptake, (ii) whether brain glucose and/or ketone uptake increases post-triheptanoin, and (iii) whether the increase in brain fuel uptake post- triheptanoin occurs in brain regions that exhibited a glucose uptake deficit pre-triheptanoin. Brain fuel uptake will be quantified by dual tracer PET according to our routine protocol (Castellano et

al. 2015). Brain fuel uptake will be expressed as *cerebral metabolic rate of glucose* (CMR-G) or AcAc (CMR-A) in the units - µmol/100 g/min, as we have described recently (Castellano et al. 2015; Nugent et al. 2014a, 2014b). These are *truly quantitative units* to express brain fuel uptake. Although the calculation of CMR-G and CMR-A requires more blood samples and is more laborious that statistical methods used to measure brain fuel uptake, i.e. statistical parametric mapping, only the CMR-G and CMR-A values provide the *actual magnitude* of regional brain fuel uptake pre- and post-triheptanoin.

The image acquisition protocol for the vMRI, rsfMRI and dMRI on our 3 Tesla MRI unit will follow our established protocols (Nugent et al. 2014; Castellano et al. 2015). vMRI-, rsfMRI- and dMRI-based markers will permit us to determine whether or not low brain glucose uptake pre-intervention or the possible change in brain glucose uptake post-intervention have related structural or functional components involving changes in white matter tracts or functional connectivity involving well-defined networks, i.e. the thalamo-cortical tract linking brain regions where brain glucose uptake is clearly deteriorating in Alzheimer's disease (Castellano et al. 2015).

11. SAMPLE SIZE

As a Phase 1b study assessing a possible new use of triheptanoin, we do not have the necessary information to reliably calculate an effect size on the co-primary outcomes in this population. Hence, the sample size estimation is necessarily approximate; in our experience with analysing paired PET-FDG and ^{11}C-AcAc images in a pre- post-treatment study design, n=12 who complete the treatment should provide sufficient statistical power to observe significant differences between treatment in our co-primary outcomes (CMR-G and CMR-A) and in vMRI parameters such as regional brain volume and cortical thickness (Nugent et al. 2014a; Nugent et al. 2014b; Castellano et al. 2015). Other groups report similar effect sizes to ours for CMR-G with sample sizes of about 10 (Baker et al. 2011).

12. CONCURRENT THERAPIES

Permissible medication

If a participant is taking prescribed medication permitted by this study design, they may continue to take it throughout the duration of the study.

Rescue medication

Prior studies have shown that high doses of triheptanoin can lead to gastrointestinal events (e.g., cramping, diarrhea, loose stools) or increased weight gain. No rescue medication will be given for side effects. In case of issues with tolerability or severe gastrointestinal distress, triheptanoin will be down-titrated by 25% for three days and then titration upwards will begin again if possible.

13. COMPLIANCE WITH PROTOCOL

At each weekly visit to monitor progress and receive the next weeks' batch of triheptanoin, each participant will also provide a mid-morning blood sample 2-3 hours after having taken the first daily triheptanoin dose at breakfast. Along with other plasma metabolites, electrolytes and enzymes, plasma beta-hydroxybutyrate and medium chain acyl-carnitines will be measured as an objective indicator of compliance. Participants in whom plasma heptanoyl-carnitine is measurable on a weekly basis and who have achieved an average daily intake of ≥0.6 g/kg/d over ≥90% of the 28 day study period will be considered compliant and will be included in the data analysis.

14. STUDY WITHDRAWAL

Participants may voluntarily withdraw from the study at any time. Participation will be discontinued if the study treatment is intolerable or unsafe, or if the participant develops any medical condition that in the opinion of the investigator would put a participant at an unacceptable medical risk or would compromise a participant's ability to participate in the study. The reason for discontinuation will be documented.

Study withdrawal criteria

Participants will be discontinued from the study or receive no further study treatment if any one of the following criteria is met:

- Participant unable or unwilling to continue to comply with study protocol.
- Participant develops or has an exacerbation of any medical condition that in the opinion of the investigator would put the participant at an unacceptable medical risk or would compromise the participant's ability to participate in the study.
- Serum triglycerides higher than 2.20 mmol/L or serum total cholesterol higher than 6.2 mmol/L .
- A systolic pressure higher than 140 mmHg and a diastolic pressure higher than 90 mmHg.
- Deteriorating liver function (ALT ≥ 37 UI/L, AST ≥ 36 UI/L, total bilirubin ≥ 26 µmol/L).
- Deteriorating renal function (creatinine ≥ 92 µmol/L, glomerular filtration rate < 60 ml/min/1.73 m^2 or > 90 ml/min/1.73 m^2)
- Clinically significant changes in other laboratory result

15. STUDY MANAGEMENT

The BEAT7 Study will be conducted at the Research Center on Aging, Université de Sherbrooke, Sherbrooke, Québec, Canada. It will be registered with Health Canada and with the Clinicaltrials.gov registry. The study will be conducted in accordance with applicable guidelines and regulations for Good Clinical Practice and will be approved by the Research Center on Aging's Research Ethics Committee (REC) prior to initiation. The BEAT7 Study team will have regular weekly meetings to discuss all aspects of the study. The investigator will promptly report to the REC any new information that may adversely affect the safety of the participants or the conduct of the study.

16. SAFETY

Published clinical studies have shown that triheptanoin at 1 g/kg/d is safe for human use in both children and older adults (UX007 Investigators' Brochure; Mochel et al. 2005; Roe et al. 2009; Pascual et al. 2014). The safety of the participants will be monitored at weekly laboratory assessments (complete blood count, liver function, blood lipid profile, renal function). Participants will be asked to report adverse events (AEs) during each weekly visit. All serious AEs (SAEs) will be reported to the REC and to Health Canada. In case of intolerance, the triheptanoin dose will be down-titrated to the previous dose for three days prior to titrating back to the higher dose. If the participant can maintain triheptanoin intake at ≥60% of the intended dose, they will be invited to continue in the study; if not they will be invited to discontinue.

Adverse events (AE)

An AE is defined as any untoward medical occurrence in a study participant who is receiving an investigational product, and for which there may or may not be a causal link to the intervention. Hospitalisation for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE. An AE can therefore be any unfavourable and unintended sign (including laboratory result), symptom, or disease that is temporally associated with the investigational product, whether or not it is actually related to the investigational product. The presence of AEs will be assessed at each visit by direct questioning of the participant by a member of the BEAT7 Study team (usually the research nurse). The severity of any AE will be graded as mild, moderate or severe. The principal investigator will assess whether there is any relationship to the investigational product. All AEs will be followed up until resolved or until, in the opinion of the principal investigator, the AE is stabilized or has become chronic. The nature, severity, causality and course of the AE will be recorded in the study database using an Excel spreadsheet.

Serious adverse events (SAE)

A serious adverse event (SAE) is any untoward medical occurrence that, at any dose of the investigational product: results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, is a congenital abnormality or birth defect, or is an important medical event. Important medical events are those that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention. SAEs will be reported to the study's senior physician (Dr. Tamas Fulop) and to the REC as soon as possible.

Clinical laboratory abnormalities and other abnormal assessments as AEs and SAEs

Abnormal laboratory findings, i.e. clinical chemistry, haematology, and urinalysis, or other abnormal assessments, i.e. excessive weight gain or loss, or change in vital signs, that are judged by the investigator as clinically significant will be recorded as AEs or SAEs if they meet one of the definitions above. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with a disease reported in the medical history (unless judged by the investigator as more severe than expected for the participant's condition) or that are present or detected at the start of the study and do not worsen following start of the study, will not be reported as AEs or SAEs. The investigator will exercise medical judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Time period, frequency, and method of detecting AEs

All AEs and SAEs will be recorded from the time of consent. Each participant will be monitored regularly by the investigator and study personnel for AEs and SAEs occurring throughout the BEAT7 study.

Recording of AEs and SAEs

When an AE/SAE occurs, the principal investigator will review all documentation (hospital, laboratory, diagnostic reports) relative to the event. The investigator will then record all relevant information regarding an AE or SAE in the appropriate form. For each SAE, start and stop dates, action taken, outcome and relationship to study product (causality) must be documented. The investigator will attempt to establish a diagnosis of the event based on signs/symptoms and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms should be documented. All details of any treatments initiated due to the SAE will be recorded in the participant's notes and entered in the study database using an Excel spreadsheet as required by our REC.

Prompt reporting of SAEs

SAEs require prompt action. Once a member of the team becomes aware that an SAE has occurred, the study coordinator and investigator will be notified within one working day. The study SAE form will be completed as thoroughly as possible with all available details of the SAE, signed by the investigator or appropriately qualified designee, and transmitted to the study database and to the REC within one working day of first becoming aware of the SAE. Even if all information concerning the SAE is not yet available, notifying the investigator and the REC will not be delayed before completing the information form to the extent possible; the form will be updated as soon as possible.

The investigator will always provide an assessment of causality at the time of the initial report (see *Assessment of Causality* below). If data obtained after reporting the AE indicate that the assessment of causality was incorrect, then the SAE form will amended, signed, dated and resubmitted. The investigator will also notify the REC in accordance with requirements. The investigator and others responsible for participant care should institute any supplementary investigations of SAEs based on their clinical judgement of the likely causative factors. This may include seeking further opinion from a specialist in the field of the AE or requesting extra tests. If a study participant dies, date of death and any post-mortem findings, including histopathology will be provided if available.

The investigator will submit a report on the SAE to the REC, Health Canada and Ultragenyx as per the regulations within the stipulated timelines.

Evaluating AEs and SAEs

Assessment of Intensity: The investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of AEs and SAEs will be assigned to one of the following categories: <u>mild</u> (an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities); <u>moderate</u> (an event that is sufficiently discomforting to interfere with normal daily activities); <u>severe</u> (an event that is incapacitating and prevents normal daily activities).

Assessment of Causality: The investigator will assess the relationship between the investigational product and the occurrence of each AE or SAE. The investigator will use clinical judgement to determine this relationship. Alternative causes such as natural history of underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to consuming the investigational product will be considered and investigated. The investigator will also consult the Investigator Brochure for UX007 in making this assessment.

The causal relationship to the investigational product will be assessed using the following classifications: <u>Not related</u> (in the investigator's opinion, there was not causal relationship between

the investigational product and the AE); Unlikely (the temporal relationship between the AE and the investigational product is such that the latter is not likely to have had any reasonable association with the AE); Possible (the AE could have been caused by the participant's clinical state or the investigational product); Probable (the AE follows an reasonable temporal sequence rom the time of investigational product administration, abates upon discontinuation of the investigational product, and cannot reasonably be explained by the known characteristics of the participant's clinical state); or Definitely (the AE follows a reasonable temporal sequence from the time of investigational product administration or reappears when the investigational product is reintroduced).

There may be situation in which an SAE has occurred and the investigator has minimal information to include in the initial report. However, the investigator will always make an initial assessment of causality for every AE prior to transmission of the SAE form. The opinion of the investigator may change in light of follow-up information and the SAE form will be amended accordingly. The causality assessment is one of the criteria when determining regulatory reporting requirements.

Assessment of Expectedness: Expected (An AE the nature or severity of which is consistent with the applicable information on the investigational product for an unapproved medicinal product (i.e. Investigators' Brochure for UX007); Unexpected (An AE the nature of which is not consistent with information on the investigational product in the Investigators' Brochure for UX007).

Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator will actively follow each participant and provide further information on the participant's condition. All AEs and SAEs documented at a previous visit/contact and that are designated as ongoing, will be reviewed at subsequent visits/contacts. All AEs and SAEs will be followed up until resolution, until the condition stabilizes, until the event is otherwise explained, or until the participant is lost to follow-up. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations or consultation with other healthcare professionals.

17. DATA ANALYSIS

We routinely analyze dual tracer PET scans with Freesurfer and PMOD software (Castellano et al. 2015; Nugent et al. 2014a; Nugent et al. 2014b; Nugent et al. 2015). Image acquisition protocols and analysis pipelines to quantify brain fuel uptake and MRI-based measures (regional vMRI, rsfMRI, dMRI) will be supervised by the study co-investigators, Drs. Whittingstall and Descoteaux. All region-based brain PET and MRI analyses will undergo a $p \leq 0.05$ false discovery rate correction for multiple comparisons (Nugent et al. 2014a, 2014b). Water diffusion (diffusivity), fractional anisotropy (broadly inversely correlated to diffusivity) and apparent fiber density will be mapped along major white matter bundles, thalamo-cortical connections, as well as in regions initialized from PET-detected regions of interest. Global and regional grey matter differences for CMR-A and CMR-G pre- to post-intervention will be assessed using the general linear model framework implemented in the commercially-available SPM8 (Matlab, MathWorks, Natick, MA, USA).

Expected outcomes

For the co-primary outcomes, we can expect increased CMR-A at the end of the triheptanoin supplementation period. We have not seen increased CMR-G after four days on a high fat ketogenic

diet (Courchesne-Loyer et al. 2015), but this may still occur after 28 days on triheptanoin. We do not expect to see any cognitive changes both because of the short duration of the intervention and because of the normal cognitive status of the participants.

Interpretation of results

These results will contribute to knowing the safety of triheptanoin in older people and its effect on brain uptake of glucose and acetoacetate. We may also learn whether resting state functional connectivity or white matter integrity changes after triheptanoin. These results may provide a rationale for a future triheptanoin intervention in older people with mild cognitive impairment or Alzheimer's disease in with cognitive performance as the primary outcome.

18. DATA COLLECTION AND STORAGE

The master list with participant names and numbers and all study documents will be securely stored by the principal investigator. Data specific to the BEAT7 Study participants will be denominalized and stored electronically. Direct access to the individual denominalized data will be strictly limited to the researchers who are directly involved in the study (authorized investigators, collaborators, study coordinator, research assistant, statistician, students) and only for the purposes of the study. The BEAT7 Study will be conducted in accordance with all applicable electronic record and data capture regulations. All study records will be retained for 25 years in accordance with Health Canada regulations (C.05.012 [3]).

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