Triheptanoin for the treatment of brain energy deficit: A 14-year experience

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Review

Triheptanoin for the Treatment of Brain Energy Deficit: A 14-Year Experience

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Triheptanoin is an odd-chain triglyceride with anaplerotic properties—that is, replenishing the pool of metabolic intermediates in the Krebs cycle. Unlike even-chain fatty acids metabolized to acetyl-CoA only, triheptanoin can indeed provide both acetyl-CoA and propionyl-CoA, two key carbon sources for the Krebs cycle. Triheptanoin was initially used in patients with long-chain fatty acid oxidation disorders. The first demonstration of the possible benefit of triheptanoin for brain energy deficit came from a patient with pyruvate carboxylase deficiency, a severe metabolic disease that affects anaplerosis in the brain. In an open-label study, triheptanoin was then shown to decrease nonepileptic paroxysmal manifestations by 90% in patients with glucose transporter 1 deficiency syndrome, a disease that affects glucose transport into the brain. ^{31}P magnetic resonance spectroscopy studies also indicated that triheptanoin was able to correct bioenergetics in the brain of patients with Huntington disease, a neurodegenerative disease associated with brain energy deficit. Altogether, these studies indicate that triheptanoin can be a treatment for brain energy deficit related to altered anaplerosis and/or glucose metabo-lism.

Key words: Krebs cycle; astrocytes; GLUT1; Huntington disease; magnetic resonance spectroscopy

BACKGROUND ON THE USE OF TRIHEPTANOIN IN HUMANS

Triheptanoin

Triheptanoin is a medium-chain triglyceride of three 7-carbon fatty acids with a molecular formula of $C_{24}H_{44}O_6$. Triheptanoin is metabolized rapidly in the gut to form glycerol and heptanoate (Fig. 1). Glycerol is converted to pyruvate, and heptanoate can be further metabolized in the liver to C4-ketone bodies (3-hydroxybutyrate and acetoacetate) and C5-ketone bodies (3-hydroxypentanoate and 3-ketopentanoate) (Kinman et al., 2006; Deng et al., 2009) (Fig. 1). Unlike even-chain fatty acids metabolized to acetyl-CoA only, heptanoate can provide both acetyl-CoA and propionyl-CoA, two key carbon sources for the Krebs cycle (Fig. 1). In the brain, heptanoate and/or C5-ketone bodies are metabolized primarily through the glial Krebs cycle (Marin-Valencia et al., 2013; Hadera et al., 2014) (Fig. 2). Triheptanoin has been used clinically for over a decade in more than 200 subjects in human studies of a variety of different diseases, including about 70 children (Roe et al., 2002; Roe and Mochel, 2006; Mochel et al., 2010; Pascual et al., 2014; Mochel et al., 2016), and many of them received over 5 years of treatment. These data support the safety of triheptanoin in humans, usually when administered at a dose between 1 and 2.5 g/kg body weight depending on age.

Initial Clinical Experience with Triheptanoin

Triheptanoin was first used in patients with long-chain fatty acid oxidation disorders caused by defects in the metabolic pathway that converts long-chain fatty acids

SIGNIFICANCE

Brain energy deficit is widely associated, as a cause or a consequence, with neurodegeneration. Triheptanoin is a drug that increases energy production through the production of intermediates for the Krebs cycle, a very important process in cellular respiration. Recent work with patients affected by three rare diseases—pyruvate carboxylase deficiency, glucose transporter 1 deficiency syndrome, and Huntington disease—showed that triheptanoin can improve brain energy deficit. This is promising for other neurodegenerative disorders in which energy deficit plays an important role.

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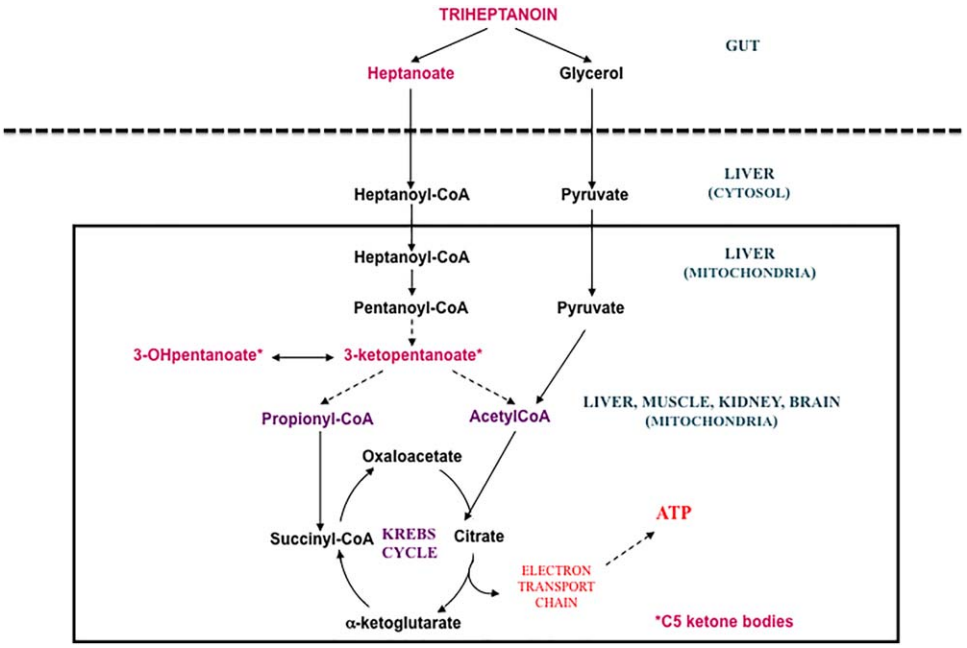

Fig. 1. Metabolism of triheptanoin. Triheptanoin is split into glycerol and three molecules of heptanoate in the gut. In the liver, glycerol is converted to pyruvate, and heptanoate can be further metabolized to C4-ketone bodies (3-hydroxybutyrate and acetoacetate) and C5-ketone bodies (3-hydroxypentanoate and 3-ketopentanoate). In the liver, or after export to the muscle, the kidney, or the brain, C5-ketone bodies are further metabolized to acetyl-CoA and propionyl-CoA, unlike even-chain fatty acids metabolized to acetyl-CoA only. In the brain, a direct uptake of heptanoate is also possible by passive diffusion (see Fig. 2).

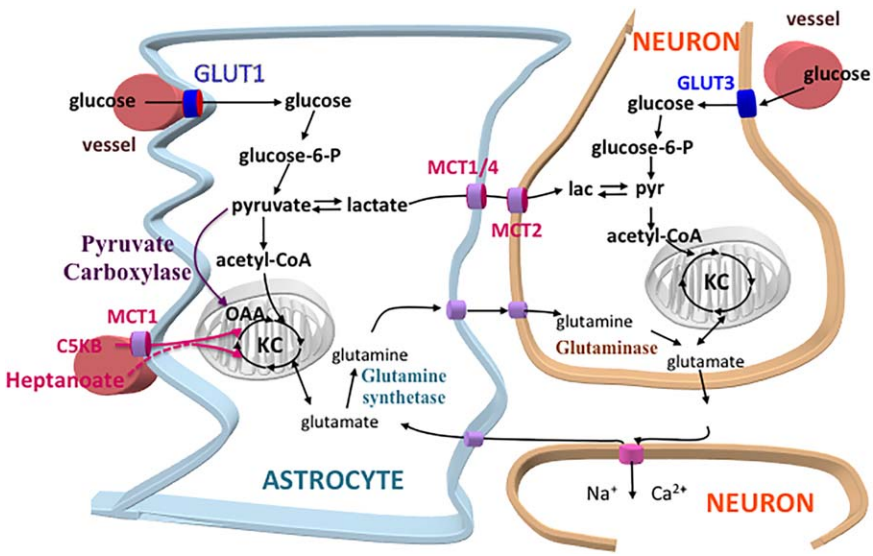

Fig. 2. Pyruvate carboxylase deficiency, GLUT1 syndrome deficiency, and treatment with triheptanoin. Illustration of astrocyte–neuron metabolic coupling, highlighting metabolic specificities from each cell type—for instance, predominant expression of pyruvate carboxylase and glutamine synthetase in astrocytes versus predominant expression of glutaminase in neurons. Heptanoate and/or C5 ketones are metabolized primarily through the glial Krebs cycle (Marin-Valencia et al., 2013; Hadera et al., 2014). Therefore, impaired astrocytic anaplerosis due to reduced oxaloacetate resulting from pyruvate carboxylase deficiency, or impaired astrocytic glucose metabolism resulting from GLUT1 deficiency, can be compensated by the provision of Krebs cycle intermediates from heptanoate and/or C5-ketone bodies.GLUT, glucose transporter; MCT, monocarboxylate transporter; glucose-6-P, glucose-6-phosphate; pyr, pyruvate; lac, lactate; C5KB, C5-ketone bodies; OAA, oxaloacetate; KC, Krebs cycle.

into energy. The standard of care is to restrict dietary fat, avoid fasting, suppress lipolysis, and use even-carbon medium-chain triglyceride and carnitine supplementation. However, patients under these therapeutic measures are still exposed to symptoms reflecting insufficient provision of energy, in particular cardiomyopathy, rhabdomyolysis, and muscle weakness. Based on the hypothesis that this energy deficiency is caused by the reduced availability of Krebs cycle intermediates required for optimal oxidation of acetyl-CoA, triheptanoin was first tested in 3 patients harboring a deficiency in very-long-chain acyl-CoA dehydrogenase and led to the rapid clinical improvement of their cardiac and muscle functions (Roe et al., 2002). A similar therapeutic benefit was later reported in 7 patients exhibiting carnitine palmitoyltransferase II deficiency (Roe et al., 2008). More recently, a retrospective analysis of 24 patients with long-chain fatty acid oxidation disorders treated for up to 12.5 years showed a significant decrease in mean hospitalization days per year and hypoglycemia event rate per year (Vockley et al., 2015). A therapeutic effect of triheptanoin has also been reported in the management of acute cardiomyopathy associated with long-chain fatty acid oxidation disorders in 10 children, including 8 infants (Vockley et al., 2016).

PYRUVATE CARBOXYLASE DEFICIENCY: A SHIFT TOWARD BRAIN ENERGY METABOLISM

The first demonstration of the possible benefit of triheptanoin for brain energy deficit came from a patient with pyruvate carboxylase (PC) deficiency (Mochel et al., 2005). PC converts pyruvate to oxaloacetate and therefore plays an important role in refilling the pool of catalytic intermediates of the Krebs cycle in peripheral tissues and in the brain. Briefly, a 6-day-old infant presented with the most severe phenotype of PC deficiency that associates hepatic failure, severe lactic acidosis, ketoacidosis, and hyperammonemia with elevated citrullinemia. Enteral administration with a formula containing triheptanoin (about 35% of total caloric intake) resulted within 24 hours in decreased lactate and lactate/pyruvate ratio, indicating improved ratio of cytosolic NADH/NAD. Triheptanoin also led to decreased plasma ammonia and citrulline, likely reflecting the increased availability of oxaloacetate, and therefore aspartate through the malate-aspartate shuttle (Mochel et al., 2005). These immediate metabolic changes were associated with the complete restoration of hepatic function in less than 48 hours. Over the following months of treatment, increased levels of C5-ketone bodies, glutamine, and γ-aminobutyric acid (GABA) were measured in the cerebrospinal fluid (CSF), associated with improved myelin synthesis on sequential brain magnetic resonance imaging (Mochel et al., 2005).

Further to the immediate correction of peripheral energy deficiency, reflected by the prompt restoration of hepatic function, the subsequent neurometabolic response in this PC-deficient infant is of particular interest considering the exclusive glial localization of PC in the brain (Fig. 2). Besides the well-known metabolic dichotomy between neurons and glial cells—that is, neurons relying highly on oxidative metabolism and astrocytes on aerobic glycolysis (Zhang et al., 2014; Volkenhoff et al., 2015)—brain energetic organization relies indeed on the narrow metabolic coupling between these different cell types. The glutamate-glutamine(-GABA) shuttle between neurons and astrocytes first illustrated such metabolic interactions more than 30 years ago with the selective expression of glutamine synthetase by astrocytes that is essential for the recycling of glutamate released from neurons in glutamatergic neurotransmission (Shank et al., 1985) (Fig. 2). More recently, a tricellular compartmentation of brain energy metabolism between neurons, astrocytes, and oligodendrocytes has been proposed—although still the subject of controversy (Hertz, 2004; Dienel, 2017)—via a lactate shuttle involving astrocytes at the synaptic level (Supplie et al., 2017) (Fig. 2) and oligodendrocytes at the axonal level (Funfschilling et al., 2012). Hence, the PC case study suggested that the astrocytic anaplerotic defect secondary to PC deficiency could be bypassed by C4- and C5-ketone bodies derived from triheptanoin, or heptanoate itself as shown with octanoate (Ebert et al., 2003). It also paved the way to research on neurometabolic diseases where the cerebral anaplerosis is deficient and/or glucose metabolism is impaired.

GLUCOSE TRANSPORTER 1 DEFICIENCY SYNDROME: A DISEASE MODEL FOR GLUCOSE AND ANAPLEROSIS DEFICIENCY

Glucose transporter 1 (GLUT1) deficiency syndrome (GLUT1-DS), characterized in 1991 by De Vivo et al., is caused by impaired glucose transport across the blood-brain barrier and into astrocytes (Fig. 2) (De Vivo et al., 1991). GLUT1-DS is usually associated with a mutation in the solute carrier family 2, member 1 (*SLC2A1*) gene, encoding the GLUT1 protein responsible for transporting glucose across the blood-brain barrier. However, genetic analysis can fail at identifying variants despite a very suggestive clinical and biochemical phenotype (Verrotti et al., 2012). Most commonly, GLUT1-DS is inherited in an autosomal dominant manner, but the vast majority of heterozygous *SLC2A1* mutations occur de novo. The laboratory hallmark of GLUT1-DS is low CSF glucose concentration—usually < 2.2 mmol/l—associated with low CSF lactate—usually < 1 mmol/l (Klepper, 2012). The low CSF lactate in most patients with GLUT1-DS (Leen et al., 2013) is thought to reflect the glucose shortage in the brain (Marin-Valencia et al., 2012) (Fig. 2). Decreased 3-O-methyl-D-glucose uptake assay is a functional measure of glucose transport across the red blood cell membrane that can very useful for the diagnosis of GLUT1-DS (Yang et al., 2011), although rarely available on a clinical basis as it implies the use of radioactivity and precautions in blood sample storage and preanalytical steps (Klepper, 2012). Recently, we evaluated a novel diagnostic test on red blood cells using flow cytometry, readily available in clinical practice, in 30 patients with

GLUT1-DS with predominant movement disorders, 18 patients with movement disorders due to other genetic defects, and 346 healthy controls (Gras et al., 2017). Our diagnostic rate was 78%, including patients with normal lumbar puncture or genetic analysis, and no false positive. This simple and rapid test opens exciting perspectives for the screening of GLUT1-DS in any patient, child or adult, presenting with cognitive impairment, epilepsy, ataxia and/or dystonia, or paroxysmal movement disorder.

Because glucose is the primary source of energy for the brain, this disorder results in a chronic state of cerebral energy deficiency. The phenotype typically comprises psychomotor retardation and permanent motor disorders, associated with paroxysmal manifestations including seizures and nonepileptic paroxysmal episodes (Leen et al., 2010). With age, patients tend to experience less seizures but more frequent paroxysmal movement disorders (Gras et al., 2014), likely reflecting dynamic changes in brain energy metabolism over years. Ketogenic diets, which provide C4-ketone bodies to the brain and compensate for the lack of glucose, represent the standard of care in GLUT1-DS (Klepper & Leiendecker, 2013). When properly administered, ketogenic diets are well tolerated and are highly effective in controlling epilepsy in GLUT1-DS, but seizures may recur (Klepper et al., 2005). Even when seizures are controlled, patients may continue to have cognitive and behavioral deficits (Klepper et al., 2002).

In patients with milder forms of the disease, paroxysmal movement disorders, especially dyskinesia, may be the main or the sole manifestations of the disease and can occur at any age (Pons et al., 2010). Ketogenic diets seem to be less efficient in nonepileptic paroxysmal manifestations, and sometimes result in more complex movement disorders (Leen et al., 2010). Moreover, many patients, especially adolescents and adults, have difficulty complying with the constraints of these long-term diets and their side effects. Therefore, triheptanoin was tested in GLUT1-DS patients with nonepileptic paroxysmal manifestations for whom a ketogenic diet was not a therapeutic option (ClinicalTrials.gov identifier: NCT02014883). Unlike ketogenic diets, which provide C4-ketone bodies and therefore acetyl-CoA only, triheptanoin provides both acetyl-CoA and propionyl-CoA. An open-label study was divided into three phases of 2 months each (baseline, treatment, and withdrawal), and eight patients with GLUT1-DS, including four children, were enrolled. During the baseline phase, GLUT1-DS patients experienced a mean of 30.8 ($\pm$ 27.7; 10–85) paroxysmal manifestations compared with a mean of 2.8 ($\pm$ 2.9; 0–7) when treated with triheptanoin ($P = 0.028$) (Mochel et al., 2016). This represented a 90% symptom reduction. Conversely, during the withdrawal phase, symptoms reappeared with a mean of 24.2 ($\pm$ 21.9; 5–63) paroxysmal manifestations (Mochel et al., 2016). Upon study completion, all patients wished to continue treatment with triheptanoin, and all but one are still treated today, almost 3 years after the study start, with very positive outcomes (personal unpublished data). Functional ^{31}P magnetic resonance spectroscopy (MRS) was performed to quantify phosphocreatine (PCr) and inorganic phosphate (Pi) within the occipital cortex during (activation) and after (recovery) a visual stimulus—see further details in the next section on Huntington disease. Under treatment with triheptanoin, the ratio of Pi/PCr improved during activation, contrasting with a deterioration of the spectroscopic profile when treatment was withdrawn (Mochel et al., 2016).

Despite the absence of a control group, the magnitude of the clinical effect combined with the spectroscopic responses argued against a placebo effect. Furthermore, another open-label study conducted in epileptic GLUT1-deficient patients showed a reduction of spike waves on electroencephalogram about 90 min after ingesting triheptanoin (Pascual et al., 2014). In a mouse model of GLUT1-DS, it was shown that triheptanoin can lead to the delivery of heptanoate to the brain, which can then be metabolized into glucose and neurotransmitter intermediates (Marin-Valencia et al., 2013). Since GLUT1-DS is primarily related to glucose shortage in the brain, these data indicate that triheptanoin can be a treatment for brain energy deficit related to altered anaplerosis and/or glucose metabolism. Nonetheless, a larger controlled study is needed to establish whether triheptanoin can be an alternative therapeutic approach in GLUT1-DS, especially for patients who object to or cannot comply with the constraints of ketogenic diets.

TARGETING BRAIN ENERGY DEFICIT IN NEURODEGENERATIVE DISEASES: HUNTINGTON DISEASE

The initial detailed description of Huntington disease (HD) was that of George Huntington, a medical practitioner of Pomeroy, Ohio, in 1872. HD is inherited as an autosomal dominant trait. In individuals with HD, a polymorphic trinucleotide repeat sequence (CAGn), near the 5′ end of the *HTT* gene, is expanded beyond the normal repeat range, leading to the translation of an expanded polyglutamine sequence in the huntingtin protein (Htt) (Huntington's Disease Collaborative Research Group, 1993). The clinical features of HD usually emerge in adulthood between 30 and 50 years of age, after which illness progresses steadily over a period of 15 to 25 years. Patients with HD may present with motor signs (chorea, bradykinesia, motor impersistence), with behavioral signs (irritability, anxiety, mood disturbance), or with both (Paulsen et al., 2008). The pathology of HD includes prominent neuronal loss and gliosis in the caudate nucleus and putamen along with regional and more diffuse atrophy. Energy metabolism has been under the scope of HD research for many years because of several observations in both patients and models of the disease. A reduction in ATP production was shown in brain of HD mice, even before the onset of motor deficits (Gines et al., 2003). Positron emission tomography studies conducted in HD patients showed that glucose consumption is reduced in the brain, providing strong evidence for hypometabolism,

especially in the basal ganglia, even in asymptomatic mutation carriers (Antonini et al., 1996). The underlying cause of this early energy deficit in HD brain is currently unknown, but impaired glycolysis (Browne & Beal, 2004), Krebs cycle (Tabrizi et al., 1999), and/or oxidative phosphorylation (Milakovic & Johnson, 2005) are likely involved. Mutant Htt was shown to decrease the expression of PGC-1α in the striatum of HD mice and patients, through transcriptional inhibition (Cui et al., 2006). PGC-1α is a transcriptional coactivator that regulates key energetic metabolic pathways, both in the brain and peripheral tissues (Lin et al., 2005). The possible role of PGC-1α in HD was initially suspected from the observation of selective striatal lesions in PGC-1α knockout mice. Downregulation of PGC-1α in HD striatum was then shown to affect mitochondrial energy metabolism (Lin et al., 2004), possibly by impairing oxidative phosphorylation (Cui et al., 2006). In addition, the inhibition of succinate dehydrogenase, by 3-nitropropionate or malonate, mimicking HD neuropathology in baboons (Palfi et al., 1996) and mice (Brouillet et al., 1998), suggests that a lack of substrates for the Krebs cycle and the respiratory chain is implicated in the energy deficit of HD brain.

Weight loss, reported in patients from the earliest stages of the disease, has been an important clinical observation related to energy deficiency in HD (Mochel et al., 2007; Aziz et al., 2008; Goodman et al., 2008). Using a multivariate statistical analysis of plasma components quantified by ^{1}H MRS, a distinctive signature was identified in HD mutation carriers that presented with hypercatabolism—that is, weight loss despite higher caloric intake, which was not explained by inflammatory processes or hormonal dysfunction. This metabolic signature was characterized by decreased levels of the branched chain amino acids (BCAAs)—valine, leucine, and isoleucine (Mochel et al., 2007)—possibly reflecting their mitochondrial oxidation to provide two key intermediates for the Krebs cycle, acetyl-CoA and succinyl-CoA. As decreased BCAA levels were also detected in the CSF of HD patients (Perry et al., 1969), BCAA oxidation may also be activated in the HD brain to compensate for the local energy deficit. Consequently, therapies aiming at providing substrates to the Krebs cycle stood as possible therapeutic approaches in HD.

The evaluation of the benefit of therapies targeting energy metabolism is not trivial in HD as there are no biochemical parameters (unlike PC) or paroxysmal symptoms (unlike GLUT1-DS) directly related to energy deficiency. Therefore, the identification of biomarkers of energy metabolism in HD is critical. Hence, in vivo ^{31}P MRS was used to study cellular energy homeostasis because it allows the detection of high-energy phosphates, such as ATP and PCr. A pilot open-label study was first conducted in six early-affected HD patients to monitor the tolerability of triheptanoin and evaluate the possible short-term efficacy on muscle metabolism (Mochel et al., 2010). Two patients developed muscle acidosis at baseline while performing a low-intensity exercise at baseline that resolved after 4 days of treatment with triheptanoin.

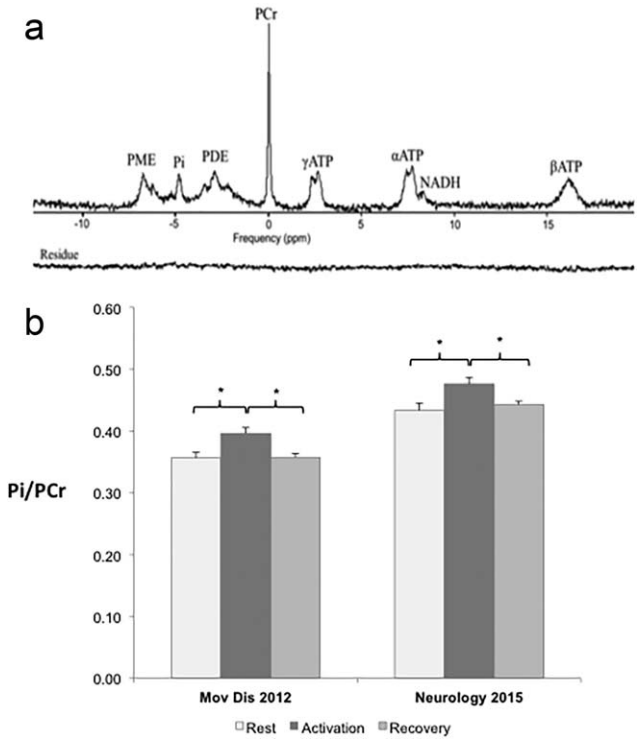

Fig. 3. ^{31}P magnetic resonance spectroscopy and brain energy metabolism. **A**: Representative ^{31}P MR spectrum from the occipital cortex of a control subject at 3T (pulse-acquire, 240 repetitions, TR = 2 sec, 8-min total acquisition time). A 2-Hz Lorentzian line broadening was applied. Compared with previous studies (Sappey-Marinier et al., 1992), the use of a surface coil adapted to the region of interest (occipital cortex), the use of a simple pulse-acquire sequence, and very good shimming all contributed to the excellent signal-to-noise ratio and spectral quality in our study. Thereby, the Pi peak was well separated from PME and PDE peaks, and therefore reliably quantified. PME, phosphomonoesters; Pi, inorganic phosphate; PDE, phosphodiesters; PCr, phosphocreatine; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide. **B**: Pi/PCr ratio before, during, and after visual stimulation in healthy controls from two independent studies (Mochel, N'Guyen, et al., 2012; Adanyeguh et al., 2015). We consistently observed a 10% increase of Pi/PCr ratio during visual stimulation compared with rest and a return to rest levels at recovery.

Measurements of muscle perfusion also suggested an effect of triheptanoin on hyperemic responses to exercise (Mochel et al., 2010). The next step was to translate ^{31}P MRS methods to the brain to identify dynamic biomarkers of energy deficiency. A visual stimulation paradigm was applied, coupled with ^{31}P MRS, to measure the concentrations of high-energy phosphates before, during, and after activation of the occipital lobe (Sappey-Marinier et al., 1992). In two independent populations of healthy controls, an increase of about 10% of Pi/PCr ratio was observed during visual stimulation compared with rest, and a return to rest levels at recovery (Mochel, N'Guyen, et al., 2012; Adanyeguh et al., 2015) (Fig. 3). This ratio is directly related to ADP levels that regulate mitochondrial oxidative metabolism; Pi/PCr ratio thus provides an index of mitochondrial oxidative regulation, as shown in

muscle (Wiener et al., 1986). Contrary to controls, HD patients at an early stage of the disease displayed no change in Pi/PCr ratios during activation and recovery, likely reflecting altered brain bioenergetics (Mochel, Durant, et al., 2012; Adanyeguh et al., 2015). This abnormal profile remained stable over 1 month without intervention but was corrected, with increased Pi/PCr ratio during visual stimulation, after 1 month of treatment with triheptanoin (Adanyeguh et al., 2015). This proof-of-concept study strongly suggested that triheptanoin is able to improve the brain metabolic profile of early-affected HD patients. Further to this short-term effect of triheptanoin on brain energy metabolism, its effect on surrogate markers of HD like caudate atrophy, which have been reported as prominent in a multicentric HD study (Tabrizi et al., 2013), and/or motor functions shall now be evaluated.

PERSPECTIVES

In the era of upcoming gene therapies for orphan diseases, triheptanoin remains a fascinating small molecule because of its peculiar composition with an odd number of carbons and, therefore, its ability to target the Krebs cycle. While initially developed for inborn errors of metabolism causing energy deficiency in peripheral tissues such as long-chain fatty acid oxidation disorders, the neurometabolic response observed in an infant with PC deficiency, although not reproduced so far (Breen et al., 2014), constituted a first step toward the exploration of the therapeutic benefit of triheptanoin for brain energy deficit with impaired Krebs cycle and/or glucose metabolism. Although it still needs to be confirmed in a larger controlled study, the data available in GLUT1-DS strongly suggest that triheptanoin can improve brain energy metabolism with overt clinical benefit. Thanks to the development of dynamic tools to assess brain energy metabolism, it also becomes possible to determine whether other neurometabolic or neurodegenerative diseases, like HD, may benefit from anaplerotic approaches with triheptanoin.

Several promising studies have been conducted in preclinical models of other neurological diseases affecting brain energy homeostasis (Willis et al., 2010; Francis et al., 2014; Hadera et al., 2014; Park et al., 2014). In the *nur*7 mouse model of Canavan disease, a leukodystrophy caused by the deficiency in aspartoacylase that hydrolyzes N-acetylaspartic acid into aspartic acid and acetate, triheptanoin reduced oxidative stress, promoted oligodendrocyte survival, and increased myelin content in the brain (Francis et al., 2014). Of note, the therapeutic benefit was much more prominent in mice treated right after birth compared with older mice (Francis et al., 2014). In male mice hemizygous for Mecp2 knockout, a model for Rett syndrome, triheptanoin increased longevity and improved motor function and social interaction together with reduced mitochondrial pathology (Park et al., 2014). In two mouse models of chronic seizures, triheptanoin exerted anticonvulsant effects (Willis et al., 2010) associated with increased Krebs cycle intermediates in the brain of the epileptic mice (Hadera et al., 2014). Trials in humans shall now evaluate the clinical benefit of triheptanoin on seizure control in idiopathic epilepsy, as well as metabolic causes of epilepsy such as GLUT1-DS. A short-term stabilization of disease progression was also reported in a pilot open-label study conducted in patients affected by adult polyglucosan body disease due to glycogen brancher enzyme deficiency (Roe et al., 2010), which must be reproduced in a randomized controlled trial.

QUESTIONS TO BE ADDRESSED

Together with the therapeutic developments of triheptanoin, important questions remain to be addressed. If the energy deficiency associated with the diseases mentioned above is prominent enough, one can expect that improving energy homeostasis will slow, or even halt, disease progression. However, such an intervention should probably be initiated at the early stages of the disease when cell loss is not too far advanced. For genetic conditions, ideally the intervention should be started before the onset of symptoms—that is, at the premanifest stage of the disease—which seems ethically acceptable considering the safety profile of triheptanoin in humans. However, the evaluation of the benefit of such intervention will remain challenging in the many neurological diseases where biochemical or clinical biomarkers of energy deficiency are not available.

Although currently not a major issue for patients, one can hope that future pharmaceutical developments will allow the identification of a more convenient formula for triheptanoin than the current liquid oil. This may be particularly useful for long-term compliance, especially in subjects with minimal or no symptoms who lead a normal socioprofessional life. Nonetheless, it is important to keep in mind that the administration of triheptanoin does require dietary adjustments, especially the replacement of a good portion of the regular fat by triheptanoin and some restrictions in sugar consumption.

Last, but not least, further work on the dose regimen of triheptanoin should be performed. In most studies, triheptanoin has been administered to provide about 35% of total caloric intake, in part based on the traditional dose of regular medium-chain triglyceride used for patients with long-chain fatty acid oxidation disorders. A number of publications also provide data on absorption and metabolism of triheptanoin in rodents when administered intravenously and orally at doses up to 40% of the recommended caloric intake (Kinman et al., 2006; Gu et al., 2010). The low heptanoate and glycerol blood concentrations during intraduodenal infusion of triheptanoin in rats indicated that the glycerol and heptanoate derived from intestinal hydrolysis was taken up and cleared by the liver (Kinman et al., 2006). Furthermore, doses higher than 40% of total caloric intake can be poorly tolerated in humans with diarrhea and nausea. In GLUT1-DS, while we aimed for a standard dose of 30% to 35% of caloric

intake, some patients seemed to do well under lower doses of 25% to 30% (personal unpublished data). Hence, titration studies will be very useful to better determine patients' individual requirements.

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CONFLICT OF INTEREST STATEMENT

Dr. Mochel holds a patent on the use of triheptanoin in Huntington disease (WO2008068230) and GLUT1-DS (WO2014093901). Dr Mochel received research support from Ultragenyx and Ipsen, and an honorarium on an advisory board from Ultragenyx.

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