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The Pharmacokinetics of Triheptanoin and Its Metabolites in Healthy Subjects and Patients With Long-Chain Fatty Acid Oxidation Disorders

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Abstract

Long-chain fatty acid oxidation disorders (LC-FAODs) are a group of life-threatening autosomal recessive disorders caused by defects in nuclear genes encoding mitochondrial enzymes involved in the conversion of dietary long-chain fatty acids into energy. Triheptanoin is an odd-carbon, medium-chain triglyceride consisting of 3 fatty acids with 7 carbons each on a glycerol backbone developed to treat adult and pediatric patients with LC-FAODs. The pharmacokinetics of triheptanoin and circulating metabolites were explored in healthy subjects and patients with LC-FAODs using noncompartmental analyses. Systemic exposure to triheptanoin following an oral administration was negligible, as triheptanoin is extensively hydrolyzed to glycerol and heptanoate in the gastrointestinal tract. Multiple peaks for triheptanoin metabolites were observed in the plasma following oral administration of triheptanoin, generally coinciding with the time that meals were served. Heptanoate, the pharmacologically active metabolite of triheptanoin supplementing energy sources in patients with LC-FAODs, showed the greatest exposure among the metabolites of triheptanoin in human plasma following oral administration of triheptanoin. The exposure of heptanoate was approximately 10-fold greater than that of beta-hydroxypentoate, a downstream metabolite of heptanoate. Exposure to triheptanoin metabolites appeared to increase following multiple doses as compared with the single dose, and with the increase in triheptanoin dose levels.

Keywords

heptanoate, LC-FAODs, pharmacokinetics, triheptanoin

Long-chain fatty acid oxidation disorders (LC-FAODs) are a group of life-threatening autosomal recessive disorders caused by defects in nuclear genes encoding mitochondrial enzymes involved in the conversion of dietary long-chain fatty acids into energy.[1] The inability to metabolize long-chain fatty acids can result in acute metabolic crises during times of increased energy demand, such as common infection or moderate exercise.[2] Acute metabolic crises may present as serious clinical manifestations including rhabdomyolysis, hypoglycemia, and cardiomyopathy.[2]

Triheptanoin is an odd-carbon, medium-chain triglyceride consisting of 3 fatty acids with 7 carbons each on a glycerol backbone developed to treat adult and pediatric patients with LC-FAODs by providing an additional energy source.[3–5] In single-arm clinical studies, triheptanoin reduced major clinical events (including hospitalizations) that affect and threaten the lives of patients with LC-FAODs and improved functional health and vitality with minimal risk.[6,7] Furthermore, triheptanoin has been studied clinically over 13 years, and the available literature provides clinical responses associated with LC-FAODs.[8–13]

Triheptanoin is extensively hydrolyzed into glycerol and heptanoate (C7 fatty acids) in the gastrointestinal (GI) tract by pancreatic lipases, and subsequently, heptanoate is absorbed through the gut wall.[14] Heptanoate can diffuse across the mitochondrial membranes and undergo a series of beta-oxidative metabolic reactions to generate acetyl coenzyme A (CoA) and

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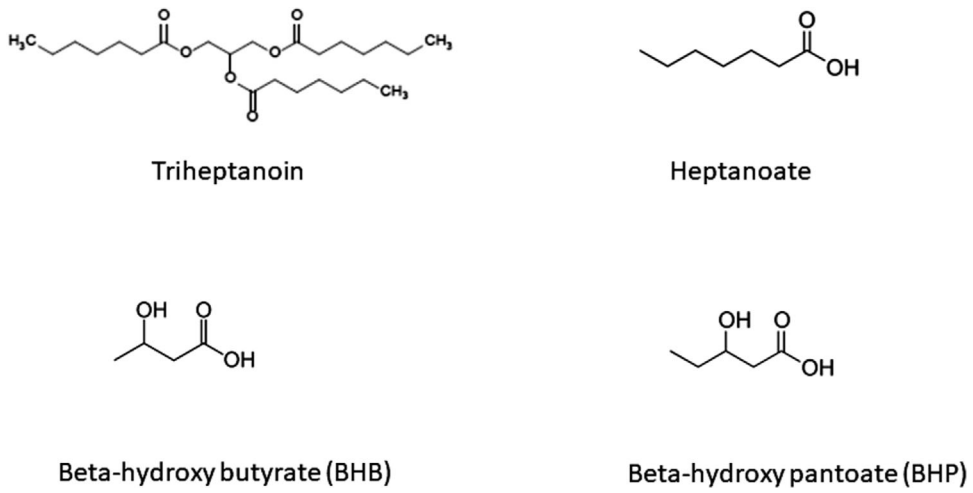

Figure 1. Chemical structures of triheptanoin and its metabolites.

propionyl-CoA, bypassing the carnitine shuttle and long-chain fatty acid oxidation enzymes that are deficient in patients with LC-FAODs.[3,4,15] Acetyl-CoA enters the tricarboxylic acid (TCA) cycle, where the acetyl group is oxidized to generate energy and carbon dioxide.[16] Propionyl-CoA is further metabolized to succinyl-CoA, which resupplies the TCA cycle intermediates (anaplerosis) that may be secondarily deficient in patients with LC-FAODs and provides substrates for gluconeogenesis.[17–19] The generation of propionyl-CoA, a critical intermediate for anaplerotic and gluconeogenic properties, is unique to triglycerides with odd-carbon fatty acids, such as triheptanoin.[18,20–26]

As beta oxidation occurs in the mitochondria, heptanoate can be metabolized to generate acetyl-CoA and propionyl-CoA in most tissues and organs with mitochondria. Beyond the beta oxidation, additional metabolic conversion of heptanoate can occur in the liver. Heptanoate can be converted to ketone bodies such as beta-hydroxybutyrate (BHB) and beta-hydroxypentoate (BHP).[27] Ketone bodies produced from the liver are released into the bloodstream and taken up by the other tissues where the ketone bodies can be used as substrates and intermediates for the TCA cycle.[28] Notably, heptanoate and ketone bodies (ie, BHB and BHP) are circulating metabolites of triheptanoin that can be quantitated in the human plasma. Other metabolites from beta oxidation (ie, acetyl-CoA and propionyl-CoA) are not released into the bloodstream but used as energy sources in the respective tissues and organs.

The pharmacokinetics (PK) of triheptanoin and circulating metabolites in human plasma were explored in 2 clinical studies (phase 1 study in adult healthy subjects, and phase 2 study in adult and pediatric subjects with LC-FAODs). Here, we present the PK characteristics of triheptanoin and circulating metabolites in healthy subjects and subjects with LC-FAODs using noncompartmental PK analyses. The chemical structures of triheptanoin and its metabolites (heptanoate, BHB, and BHP) are provided in Figure 1.

Subjects and Methods

Clinical Studies

In the phase 1 study, a total of 14 healthy adult subjects enrolled and 13 subjects completed the study. On day 1 of the study, subjects were randomized 1:1 to either a powder-to-oil or oil-to-powder treatment sequence and began dosing on day 1 of period 1. Each subject completed 4 treatment periods with triheptanoin administered as (1) single dose (SD) and multiple doses (MDs) as powder at 1.25 g/kg/d, (2) SD and MDs as oil at 1.25 g/kg/d, (3) SD as powder at 1.5 g/kg/d, and (4) SD as oil at 1.5 g/kg/d. The dose level of 1.25 g/kg/d corresponds to ≈30% of daily caloric intake (DCI) in healthy adults. During the SD treatment period, healthy subjects received 25% of the total daily dose in the assigned formulation (ie, 0.3125 g/kg, 25% of total daily dose of 1.25 g/kg/d; 0.375 g/kg, 25% of total daily dose of 1.5 g/kg/d). During the MD treatment period, the total daily dose was divided into 4 equal doses, and subjects received triheptanoin 4 times per day. Triheptanoin was orally administered either mixing with drinks (powder) or foods (oil) to reduce GI-related adverse events. As the powder formulation has not been used in subsequent clinical studies, the PK analyses of triheptanoin powder and its metabolites are not presented here. PK blood samples were collected from all study participants, and PK was characterized in a total of 13 subjects in the phase 1 study. Subjects were admitted to the clinical unit on day –1 and remained over the duration of the study. Standardized meals were provided, and dietary intake was monitored and recorded throughout the study.

Table 1. Overview of Clinical Studies Included in the Current PK Analyses

Study Title	Dose Regimen	Number of Subjects With PK	Planned PK Sampling
A phase 1, randomized, crossover, pharmacokinetic, safety and tolerability study of UX007 formulations in healthy volunteers	Single dose: 0.3125 g/kg (equivalent to 1.25 g/kg/d, close to 30% of daily caloric intake) or 0.375 g/kg (equivalent to 1.5 g/kg/d) Multiple dose: 0.3125 g/kg (equivalent to 1.25 g/kg/d) Triheptanoin administered with foods or drinks	13 adult healthy volunteers (≥18 years of age)	Single dose (days 1, 5, 9, and 11) PK samples: before dosing; after dosing (end of breakfast meal); and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 4, 6, and 8 h after dosing Multiple doses (days 3 and 7) PK samples: before dosing; after dosing (end of bedtime meal); and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 4, 6, and 8 h after dosing
An open-label long-term safety and efficacy extension study in subjects with long-chain fatty acid oxidation disorders (LC-FAOD) previously enrolled in UX007 or triheptanoin studies[a]	Target dose range of 25%-35% of daily caloric intake Triheptanoin administered with foods or by gastronomy tube	PK substudy: 11 adult and pediatric patients with LC-FAODs[b]	PK measurements on 2 consecutively scheduled clinic visits before dosing (within 15 min before start of meal) and at 0.5, 1.5, 2, and 4 h (±5 min) after finishing the meal

LC-FAOD, long-chain fatty acid oxidation disorder; PK, pharmacokinetic.
[a] Vockley J, Burton V, Berry G, et al. *J Inherit Metab Dis*. 2020. doi:10.1002/jimd.12313
[b] A total of 75 patients with LC-FAODs participated in the clinical study. Eleven subjects joined PK substudy and had evaluable PK data.
Note. The plasma concentrations of triheptanoin metabolites were expressed as micro-molar concentration considering mass conversion with molecular weight where molecular weights of triheptanoin, heptanoate, beta-hydroxybutyrate, and beta-hydroxypentoate are 428.6, 130.2, 104.1, and 118.1, respectively.

In the phase 2 study, subjects with LC-FAODs who had participated in prior clinical studies treated with triheptanoin or who had failed conventional therapy with documented severe unmet needs were eligible to enroll for the study. The treatment period was 1 to 5 years. A total of 75 subjects were enrolled in the study, of which 24 subjects had completed the previous phase 2 study, 31 subjects enrolled with previous triheptanoin treatment from investigator-sponsored trials or other studies, and 20 subjects enrolled as triheptanoin naïve. Triheptanoin dosing was titrated to a target dose of 25% to 35% of total DCI or maximum tolerated dose. Triheptanoin was orally administered through mixing with foods or drinks, or by gastrostomy tube. PK samples were collected in adult and pediatric subjects with LC-FAODs who had participated in the PK substudy, and PK was characterized in a total of 11 subjects in the phase 2 study.

All subjects provided written informed consent to participate in the clinical studies. Study protocols were approved by the institutional review boards at each participating site. These clinical studies were conducted in compliance with the Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The description of clinical studies, dosing regimen, and sample collections is provided in Table 1. The final protocols, amendments, and informed consent documentation were reviewed and approved by the institutional review board at each study center. The names and location of the individual institutional review boards are provided in Table S1.

Pharmacokinetic Sampling and Bioanalytical Analyses

In the phase 1 study, PK samples were collected in all subjects who had participated in the study. Plasma PK samples were collected before dosing; at the end of a meal; and 0.25, 0.75, 1, 1.5, 2, 2.5, 4, 6, and 8 hours after dosing at the respective treatment period. In the phase 2 study, PK samples were collected in subjects with LC-FAODs who had participated in the PK substudy. Plasma PK samples were collected before dosing and 0.5, 1.5, 2, and 4 hours after dosing at 2 consecutively scheduled clinic visits.

The plasma samples were analyzed for triheptanoin and its metabolites (heptanoate, BHB, and BHP) by validated bioanalytical methods. The lower limit of quantitation of the analytes was 0.2, 1.0, 8.0, and 1.0 μM for triheptanoin, heptanoate, BHB, and BHP, respectively. The upper limit of quantitation of the analytes was 10, 250, 800, and 100 μM for triheptanoin, heptanoate, BHB, and BHP, respectively. These methods were validated for selectivity, sensitivity, carryover, linearity, precision, accuracy, recovery, matrix effect, and stability and reinjection reproducibility according to US Food and Drug Administration guidelines.

For triheptanoin, immediately after blood collection and plasma separation, 200-μL plasma sample aliquots were stabilized by adding 10 μL of 85% phosphoric acid. Each 25-μL aliquot of calibration standard, quality control, or study sample was mixed with 50 μL of working internal standard solution (glycerol trihexanoate, 5.0 μM). The sample was vortexed, and 400 μL of hexane/isopropanol alcohol (66.7/33.3, v/v) was added. The sample was vortexed and centrifuged, and 100 μL of the resulting supernatant was transferred to a clean 96-well plate. The supernatant was evaporated from the plate and reconstituted with 100 μL of methanol, then vortexed and centrifuged. The supernatant was then transferred to a clean 96-well plate and centrifuged, and an aliquot was injected onto a liquid chromatography–tandem mass spectrometry (LC-MS/MS) system for analysis. The liquid chromatography system used an XBridge C18 column, 2.1 × 50 mm (3.5 μm particle size; Waters Corporation, Milford, Massachusetts) with an isocratic flow consisting of 20 mM ammonium formate in water and methanol at a flow rate of 0.75 mL/min. The analyte and internal standard were detected using an API 5000 triple quadrupole LC-MS/MS system (AB Sciex, Framingham, Massachusetts) equipped with an electrospray ionization (TurboIonSpray) source operated in the positive ion mode. The multiple reaction monitoring (MRM) transitions of the respective [M+H] + ions were used to monitor triheptanoin and internal standard glycerol trihexanoate. Transition monitored retention time for analyte triheptanoin was *m/z* 446 → 113 0.7-1.0 minutes and for internal standard glycerol trihexanoate was *m/z* 404 → 99 0.4-0.6 minutes.

For analysis of heptanoate, BHB, and BHP, due to their endogenous nature, calibration standards were prepared in a surrogate matrix consisting of 5% bovine serum albumin solution in Dulbecco's phosphate buffered saline. Human plasma containing the anticoagulant (K2-ethylenediaminetetraacetic acid or Na-heparin) was mixed with internal standards (beta-hydroxybutyric acid-d4, beta-hydroxypentanoic acid-d3, and heptanoic acid-d3) and subjected to protein precipitation using 200 μL of 1% formic acid in methanol in a 96-well plate. Following centrifugation, the supernatant was transferred to a 96-well plate with prerinsed glass inserts and then injected twice onto an Agilent 1290/ QTrap 5500 LC-MS/MS system (AB Sciex) equipped with 2 ultra-high-performance liquid chromatography C18 columns, one for heptanoate separately and the other combined for BHB and BHP analytes. For heptanoate, mobile phase A was 0.2% ammonium bicarbonate in water and mobile phase B was acetonitrile : methanol (1:1). For BHB and BHP, mobile phase A was 0.01% formic acid in water and mobile phase B was acetonitrile:methanol (1:1) at a flow rate of 0.7 mL/min. The MRM transitions of the respective [M+H] + ions were used to monitor heptanoate, BHB, and BHP and their labeled internal standards. Transition monitored retention time for heptanoate was *m/z* 129 → 129-0.9 minutes; internal standard heptanoate-d3: *m/z* 132 → 132-0.9 minutes, for BHB was *m/z* 103 → 59-0.9 minutes; and internal standard BHB-d4: m/z 107 → 59-0.9 minutes, for BHP was *m/z* 117 → 59-1.2 minutes, internal standard BHP-d3: m/z 120 → 59-1.2 minutes. The peak areas of the respective parent ion (heptanoate; pseudo-MRM mode) and parent-to-product ion transitions (beta-hydroxybutyric acid and beta-hydroxypentanoic acid; MRM mode) were measured against the peak area of the parent or product ions (parent for heptanoate) of the corresponding internal standards. Quantitation was performed using a weighted linear least squares regression analysis with a weighting of 1/x generated from calibration standards prepared immediately before each run. For triheptanoin, heptanoate, BHB, and BHP, intra- and interrun precision and accuracy were <10% for low, medium, and high quality controls for both K2-ethylenediaminetetraacetic acid and Na-heparin validated methods.

Incurred sample reanalysis was performed for 10% of the total samples for both studies to assess the reproducibility of the assays for heptanoate, BHB, and BHP. Prespecified acceptance criteria were set to achieve at least a 67% incurred sample reanalysis sample; results must be within ±20% of the original sample result for each analyte. All analytes met the prespecified acceptance criteria. Due to results obtained for triheptanoin that were below the limit of quantitation, no incurred sample analysis was performed.

The plasma concentrations of triheptanoin metabolites were expressed as micromolar concentration considering mass conversion with molecular weight using the following equation:

$$\mu M = \mu g/mL \times \frac{1000}{molecular\ weight}$$

Table 2. Summary of Demographic Characteristics in Healthy Subjects Enrolled in Phase 1 Study

Demographics	Total (N = 14)
Sex, n (%)	
Male	6 (43)
Female	8 (57)
Race, n (%)	
White	11 (79)
Black	2 (14)
Others	1 (7)
Age, y	
Mean (standard deviation)	38.0 (11.9)
Median (min, max)	37.0 (21.0, 54.0)
Weight, kg	
Mean (standard deviation)	75.0 (13.0)
Median (min, max)	76.4 (57.4, 95.9)

where molecular weights of triheptanoin, heptanoate, BHB, and BHP are 428.6, 130.2, 104.1, and 118.1, respectively.

Noncompartmental PK Analysis

PK parameters were derived by noncompartmental analyses using a validated Phoenix WinNonlin v 7.0 (Certara, Princeton, New Jersey) based on the individual plasma concentration–time profiles. Actual time relative to the dosing time was used to derive PK parameters. Since BHB is not a unique metabolite of heptanoate but an endogenous ketone body, PK analyses were conducted after subtracting the baseline level in each subject. If baseline levels were not available, no PK analyses were conducted for BHB.

Results

Pharmacokinetics of Triheptanoin and Its Metabolites in Healthy Subjects

Key baseline characteristics of healthy subjects enrolled in the phase 1 study are provided in Table 2. Men and women were almost evenly distributed in the study (6 men vs 8 women). Subjects were mostly White (79%), and median age was 37 years (range, 21-54). Age and body weights by sex are provided in Table S2.

The majority of plasma concentrations for triheptanoin were below the lower limit of quantitation; therefore, further PK analysis was not conducted for triheptanoin. The PK parameters for triheptanoin metabolites are provided in Table 3. The median time to reach peak concentration (t_{max}) ranged from 0.7 to 1.4 hours for heptanoate following either SD or MD administrations of triheptanoin. Similar t_{max} was observed for BHB and BHP. Generally, multiple peaks for heptanoate were observed in the plasma following the SD administration of triheptanoin, as depicted in Figure 2. Multiple peaks were also observed for BHB and BHP. The median time to the first peak of heptanoate ranged from 0.5 to 1.2 hours, generally coinciding with the time when breakfast meals were consumed. A second peak was frequently observed at 4 hours after dosing, coinciding with the time when the lunch was served in the study participants. Due to multiple peaks, the terminal elimination phase cannot be adequately determined with PK samples collected up to 8 hours after dosing. Thus, the terminal half-life was not estimated for triheptanoin metabolites.

Apparent accumulations were observed for triheptanoin metabolites following MD administrations, as shown in Table 3. The exposure of triheptanoin

Table 3. PK Parameters of Triheptanoin Metabolites Following Either a Single Dose or Multiple Doses of Triheptanoin in Healthy Subjects

Triheptanoin Dose (g/kg/d)	Triheptanoin Metabolites	C_{max}, μM, Mean ± Standard Deviation	T_{max}, h, median (range)	Time to First Peak, h	$AUC_{0\text{-last}}$, (μM • h) Mean ± Standard Deviation
1.25, SD	Heptanoate	179 ± 145	0.7 (0.4-6.5)	0.5 (0.4-1.0)	337 ± 223
n = 13	BHB	141 ± 142	0.7 (0.4-4.4)	0.7 (0.4-4.4)	180 ± 207
	BHP	20.2 ± 16.8	0.7 (0.4-6.4)	0.7 (0.4-1.2)	42.5 ± 42.6
1.5, SD	Heptanoate	259 ± 134	1.2 (0.4-8.3)	0.8 (0.4-6.4)	569 ± 189
n = 13	BHB	317 ± 219	0.7 (0.0-1.4)	0.5 (0.0-0.9)	408 ± 412
	BHP	41.3 ± 20.2	2.9 (0.7-6.4)	0.9 (0.5-6.4)	116 ± 47.4
1.25, MD	Heptanoate	320 ± 163	1.4 (0.0-8.4)	1.2 (0.0-2.4)	790 ± 346
n = 13	BHB	238 ± 122	1.4 (0.0-8.4)	0.7 (0.0-4.4)	447 ± 386
	BHP	45.4 ± 25.0	1.9 (0.0-8.4)	0.4 (0.0-1.9)	152 ± 59.6

$AUC_{0\text{-last}}$, area under the plasma concentration–time curve from time 0 to the last quantifiable concentration; BHB, beta-hydroxybutyrate; BHP, beta-hydroxypentanoate; C_{max}, maximum concentration; MD, multiple dose; SD, single dose.

The dose level in the SD phase was equivalent to 25% of the total daily dose. In the MD phase, triheptanoin was divided into 4 equal doses per day. The plasma concentrations of triheptanoin metabolites were expressed as micro-molar concentration considering mass conversion with molecular weight where molecular weights of triheptanoin, heptanoate, BHB, and BHP are 428.6, 130.2, 104.1, and 118.1, respectively.

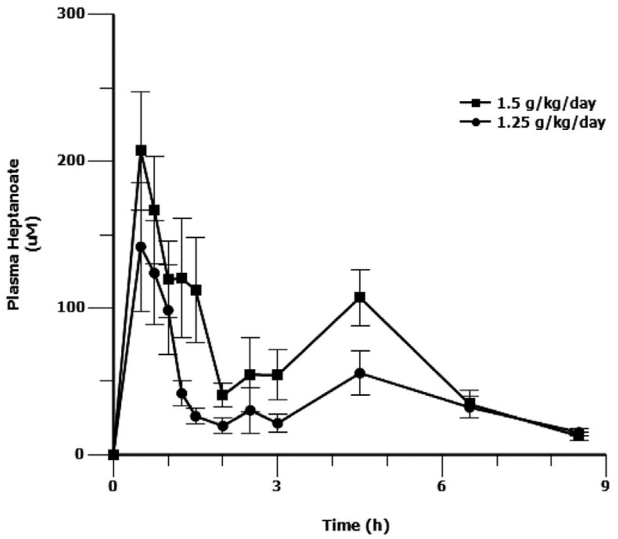

Figure 2. Concentration-time profiles of heptanoate following a single dose of triheptanoin in healthy subjects. Values below the lower limit of quantitation were considered 0 for this graph. Error bar represents a standard error. The plasma concentrations of triheptanoin metabolites were expressed as micro-molar concentration considering mass conversion with molecular weight where molecular weights of triheptanoin, heptanoate, beta-hydroxybutyrate, and beta-hydroxypentoate are 428.6, 130.2, 104.1, and 118.1, respectively.

metabolites increased with increasing dose levels from 1.25 g/kg/d to 1.5 g/kg/d. Due to a narrow dose range tested in the study, dose-proportionality for the exposure of triheptanoin metabolites cannot be evaluated appropriately. Among the metabolites of triheptanoin, plasma exposure was the greatest for heptanoate, followed by BHB and BHP.

Following the administration of triheptanoin, the plasma concentration levels of C-3, C-5, and C-7 acylcarnitine conjugates increased compared to the baseline and gradually decreased over time, as described in Figure S1.

Overall, 12 of 14 subjects (85.7%) experienced a total of 43 treatment-emergent adverse events (TEAEs) during the study. The majority of TEAEs were mild in severity and were determined by the investigator to be related to triheptanoin. The most frequent TEAEs reported in ≥2 subjects included constipation, nausea, flatulence, headache, and dermatitis, as presented in Table 4. The safety findings in healthy subjects were consistent with the known safety profile of triheptanoin reported elsewhere.[29]

Pharmacokinetics of Triheptanoin and Its Metabolites in Subjects with LC-FAODs

Key baseline characteristics of adult and pediatric subjects with LC-FAODs enrolled in the PK substudy are provided in Table 5. Men and women were almost evenly distributed in the study (6 men vs 5 women), and most subjects were White (82%). Notably, triheptanoin dose levels adjusted by body weight in pediatric subjects with LC-FAODs were ≈50% higher than those in adult subjects with LC-FAODs.

Table 4. Summary of TEAEs in ≥2 Subjects

System Organ Class/Preferred Term	Subjects With TEAE, n (%)
Any TEAE	12 (85.7)
GI disorders	
Constipation	8 (57.1)
Nausea	6 (42.9)
Flatulence	3 (21.4)
Nervous system disorders	
Headache	2 (14.3)
Skin disorders	
Dermatitis	2 (14.3)

GI, gastrointestinal; TEAE, treatment-emergent adverse event.

In this study, PK samples were collected only at steady state. Thus, PK parameters were derived at steady state, and the accumulation of triheptanoin metabolites cannot be investigated. No measurable concentrations were observed in plasma for triheptanoin; hence, further PK analysis was not conducted. This result was consistent with the clinical study conducted in healthy subjects. The PK parameters for triheptanoin metabolites at steady state are provided in Table 6. The median t_{max} ranged from 0.7 to 1.6 hours for heptanoate and from 3.0 to 3.9 hours for BHP following MD administrations of triheptanoin. The PK parameters for BHB were not determined because the baseline level before the start of treatment was not measured. Since BHB is an endogenous ketone body, the correction from baseline was necessary to adequately evaluate the increase of BHB following the administration of triheptanoin. The geometric mean plasma exposures (maximum concentration and area under the plasma concentration–time curve from time 0 to the last quantifiable concentration) at steady state were ≈10-fold higher for heptanoate compared to BHP, which is consistent with those in healthy subjects. For all triheptanoin metabolites, the terminal elimination phase cannot be characterized because the sample collection was limited up to 4 hours after dosing and did not capture the adequate elimination phase, as presented in Figure 3.

Mean plasma exposures of triheptanoin metabolites at steady state were approximately 2-fold greater in the pediatric subjects with LC-FAODs compared to adult subjects with LC-FAODs, as shown in Table 6. However, the direct comparison may be biased because the

Table 5. Summary of Demographic Characteristics and Triheptanoin Dose Levels in Adult and Pediatric Subjects With LC-FAODs in Phase 2 PK Substudy

	Pediatric Subjects (N = 6)	Adult Subjects (N = 5)	Total (N = 11)
Sex, n (%)			
Male	4 (67)	2 (40)	6 (55)
Female	2 (33)	3 (60)	5 (45)
Race, n (%)			
White	4 (67)	5 (100)	9 (82)
Black	1 (17)	0 (0)	1 (9)
Others	1 (17)	0 (0)	1 (9)
Age, y			
Mean (standard deviation)	12.0 (3.21)	31.4 (17.0)	20.8 (15.2)
Median (min, max)	12.1 (7.56, 16.9)	23.5 (18.2, 62.1)	16.9 (7.56, 62.1)
[1] Weight, kg			
Mean (standard deviation)	61.8 (22.7)	80.1 (12.4)	70.1 (20.3)
Median (min, max)	63.1 (33.8, 94.8)	76.0 (65.7, 94.0)	72.7 (33.8, 94.8)
[2] Dose, g/kg			
Mean (standard deviation)	0.358 (0.164)	0.250 (0.0293)	0.309 (0.130)
Median (min, max)	0.298 (0.154, 0.591)	0.258 (0.217, 0.280)	0.280 (0.154, 0.591)
Total daily dose, g/kg/d			
Mean (standard deviation)	1.34 (0.284)	0.862 (0.278)	1.12 (0.366)
Median (min, max)	1.24 (1.02, 1.72)	0.868 (0.443, 1.12)	1.12 (0.443, 1.72)

LC-FAODs, long-chain fatty acid oxidation disorders; PK, pharmacokinetic.
[1] Weight when PK samples were collected.
[2] Dose when PK samples were collected.

Table 6. PK Parameters of Triheptanoin Metabolites at Steady State Following Multiple Doses of Triheptanoin in Subjects With LC-FAODs

Triheptanoin Metabolite	Age Group	C_{max}, μM, Mean ± Standard Deviation	t_{max}, h, Median (Range)	$AUC_{0\text{-last}}$ (μM • h), Mean ± Standard Deviation	C_{max}/Dose [μM/(g/kg)], Mean ± Standard Deviation	$AUC_{0\text{-last}}$ /Dose (μM • h/(g/kg), Mean ± Standard Deviation
Heptanoate	Pediatrics (n = 6)	357 ± 402	0.7 (0.5-4.2)	668 ± 775	943 ± 672	1711 ± 1312
	Adults (n = 5)	140 ± 66.5	1.6 (0.6-4.0)	288 ± 213	580 ± 289	1170 ± 829
BHP	Pediatrics (n = 6)	25.6 ± 18.0	3.0 (1.5-4.2)	49.7 ± 39.1	72.5 ± 55.2	136 ± 108
	Adults (n = 5)	17.4 ± 7.92	3.9 (0.6-4.1)	29.8 ± 16.1	70.4 ± 31.8	120 ± 60.5

$AUC_{0\text{-last}}$, area under the plasma concentration–time curve from time 0 to the last quantifiable concentration; C_{max}, maximum concentration; BHB, beta-hydroxybutyrate; BHP, beta-hydroxypentanoate; LC-FAODs, long-chain fatty acid oxidation disorders; t_{max}, time to maximum concentration.
The plasma concentrations of triheptanoin metabolites were expressed as micro-molar concentration considering mass conversion with molecular weight where molecular weights of triheptanoin, heptanoate, BHB, and BHP are 428.6, 130.2, 104.1, and 118.1, respectively.

body weight–adjusted dose levels in pediatric subjects with LC-FAODs were different from those in adult subjects with LC-FAODs. The mean exposures normalized to the body weight–adjusted dose levels (ie, area under the plasma concentration–time curve from time 0 to the last quantifiable concentration/dose) in pediatric subjects with LC-FAODs was ≈40% and 10% greater than those in adult subjects with LC-FAODs for heptanoate and BHP, respectively. Safety findings of 11 subjects in the study were consistent with the known safety profile of triheptanoin and were published previously.[29]

Discussion

The systemic exposure of triheptanoin was negligible in both healthy subjects and subjects with LC-FAODs. Typically, dietary lipids (triglycerides) break down into fatty acids by pancreatic lipases in the GI tract.[30] As a triglyceride with medium-chain fatty acids, triheptanoin is hydrolyzed to glycerol and heptanoate by pancreatic lipases in the intestines, and subsequently heptanoate is absorbed into the body. Therefore, the minimal systemic exposure of triheptanoin is anticipated. Similar to other medium-chain fatty acids,

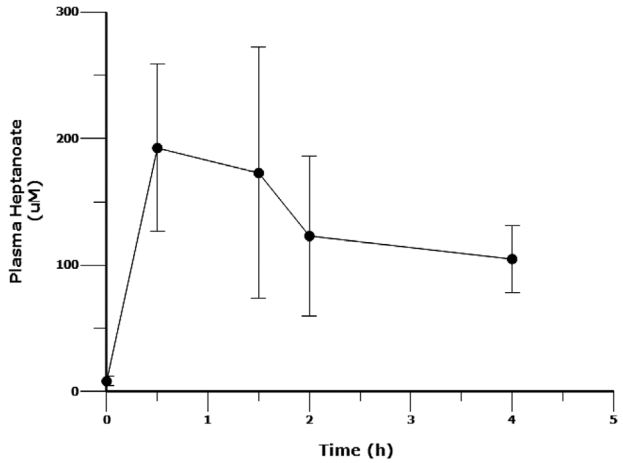

Figure 3. Concentration-time profiles of heptanoate following multiple doses of triheptanoin in subjects with long-chain fatty acid oxidation disorders. Values below the lower limit of quantitation were considered 0 for this graph. Error bar represents a standard error. The plasma concentrations of triheptanoin metabolites were expressed as micro-molar concentration considering mass conversion with molecular weight where molecular weights of triheptanoin, heptanoate, beta-hydroxybutyrate, and beta-hydroxypentoate are 428.6, 130.2, 104.1, and 118.1, respectively.

heptanoate can be absorbed from the GI tract by simple diffusion without binding to carrier proteins.[31]

Following the oral administration of triheptanoin, multiple peaks for heptanoate were observed in the human plasma. Similar observations have been reported previously in a clinical study with LC-FAODs.[14] The first peak occurred over the time of the first meal (ie, breakfast), and the second peak occurred at ≈4 hours after dosing, which coincided with the time that lunch was served to the healthy subjects in the clinic. These results suggest that the food may stimulate the bile salts and pancreatic lipases to enhance the digestion and hydrolysis of triheptanoin. In clinical studies, triheptanoin was recommended to administer with foods to reduce GI-related adverse events. Based on the current PK results, it appears that the current recommendation to administer triheptanoin with foods may be able to increase the oral bioavailability of heptanoate by enhancing the digestion and hydrolysis of triheptanoin in the GI tract.

Heptanoate showed the greatest exposure among the metabolites of triheptanoin in the human plasma following the oral administration of triheptanoin. The systemic exposure of heptanoate is approximately 10-fold greater than BHP, a downstream metabolite of heptanoate. As a pharmacologically active metabolite with the greatest systemic exposure, heptanoate is considered the major contributor to supplementing energy sources in patients with LC-FAODs following the treatment of triheptanoin. BHB is an endogenous ketone body converted from acetyl-CoA, which can be generated from multiple different biochemical pathways.[32] Therefore, BHB is not a unique metabolite converted from heptanoate. As heptanoate can be metabolized to acetyl-CoA by the beta-oxidation, the increase of BHB from the baseline could be indirect evidence that acetyl-CoA is generated from heptanoate following the oral administration of triheptanoin. BHP is converted from propionyl-CoA, which is formed by the beta-oxidation from the odd-chain fatty acids such as heptanoate.[4] Thus, BHP is considered a unique metabolite for heptanoate. These ketone bodies are released from the liver and taken up by other tissues and organs to be used as energy sources.[4]

Apparent accumulations were observed for triheptanoin metabolites following multiple dose administrations. The observed accumulation may be due to the potential time-dependent changes in the clearance of triheptanoin metabolites and/or a diurnal variation caused by the time differences of the PK sampling between SDs and MDs in the phase 1 clinical study. Blood samples were collected in the morning following the administration of triheptanoin with breakfast during the SD phase, but those samples were collected in the night following the administration of triheptanoin with bedtime meals during the MD phase.

Greater exposure of triheptanoin metabolites in pediatric subjects with LC-FAOD compared to adults with LC-FAODs appears to be related to body weight–adjusted dose levels. The differences of body weight–adjusted dose levels were caused by an individualized dosing regimen targeting 25% to 35% of DCI, which was dependent upon sex, weight, height, and age. Generally, body weight–adjusted dose levels were greater in pediatric subjects than adults because DCI tends to be higher in pediatric subjects, which is necessary for the appropriate growth and development over the pediatric age range. After normalizing by weight-based doses, the observed exposure of triheptanoin metabolites in subjects with LC-FAODs was comparable between adult and pediatric patients, suggesting that the overall absorption and disposition of triheptanoin and circulating metabolites were not affected by age.

In conclusion, PK characteristics of triheptanoin metabolites were similar between healthy subjects and patients with LC-FAODs. Heptanoate appears to be a major contributor to supplementing energy sources in patients with LC-FAODs. No differences in the absorption and disposition of triheptanoin metabolites were observed between adult and pediatric patients with LC-FAODs.

Conflicts of Interest

S.K.L., M.G., and K.M. are employees of Ultragenyx. J.S. was an Ultragenyx employee at the time these studies were conducted.

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