Parenteral and enteral metabolism of anaplerotic triheptanoin in normal rats

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The classical chronic treatment of long-chain FOD involves providing about one-third of the calories as triheptanoin (22). The catabolism of heptanoate yields anaplerotic propionyl-CoA in addition to acetyl-CoA. It was hypothesized that part of the energy deficit in FOD patients results from a decrease in the concentration of citric acid cycle intermediates in muscle and heart cells. These intermediates carry the carbons of acetyl groups as they are oxidized. The treatment with triheptanoin resulted in a rapid improvement of muscle and cardiac function in the three long-chain FOD patients described in Ref. 22, as well as in other patients who are presently involved in clinical trials (Roe CR, unpublished observations). Also, treatment with triheptanoin improved for 6 mo the condition of a patient with almost complete deficiency of liver pyruvate carboxylase (a major contributor to hepatic anaplerosis) before she succumbed to an acute episode of gastroenteritis (17).

In the liver, propionyl-CoA derived from heptanoate oxidation has two main fates. First, it is gluconeogenic via succinyl-CoA and part of the citric acid cycle reactions. Second, it is converted to the C5-ketone bodies β-ketopentanoate and β-hydroxybutyrate (BHB). In peripheral tissues, the C5-ketone bodies are analogous to the C4 acetoacetate (AcAc) and β-hydroxybutyrate (BHB). In peripheral tissues, the C5-ketone bodies are converted to acetyl-CoA and anaplerotic propionyl-CoA by the combined action of 1) β-oxidation of heptanoate and 2) utilization of C5-ketone bodies formed in the liver. The goal of the present study was to test in normal rats the metabolism of a triheptanoin preparation that might be used in the future for the treatment of acute long-chain FOD decomposition, via either intravenous or enteral infusion. An emul-
sion of triheptanoin was infused intravenously (iv) or intraduodenally (id), the latter to delineate the role of first-pass metabolism of the substrate through the portal drainage system. Our data show that the route of administration has a major impact on the distribution of triheptanoin metabolites in systemic blood.

MATERIALS AND METHODS

Materials

[U-13C6]glucose, [6,6-2H2]glucose, [3-13C]glycerol, [U-13C3]glycerol, [ω-1H]heptanoic acid, [1-15N]O2, Na2O2H, sodium borodeuteride (NaBD4), and general chemicals were purchased from Isotec/Sigma-Aldrich. Pentafluorobenzyl bromide was purchased from Pierce. An internal standard of R,S-β-hydroxy-13CH3-pentanoate was prepared by incubating ethyl β-ketopentanoate in 2H2O plus Na2O2H, followed by reducing β-keto-13CH3-pentanoate with sodium borodeuteride (16). A 10% sterile emulsion of triheptanoin was kindly donated by Sasol Germany. As per Sasol’s quality control data, the heptanoic acid used to synthesize triheptanoin contained 99.5% heptanoic acid, with traces of hexanoic, octanoic, and decanoic acids. The triheptanoin used to prepare the emulsion contained 97.5% triheptanoic acid, with the remaining 2.5% being monoester and diester. The emulsion was stabilized with 1% Invitro 2020 (Sasol), a food-approved emulsifier prepared by interesterification of a mixture of compounds with the following weight composition: 14% glycerol, 18.5% tartaric acid, 15% acetic acid, 4.5% palmitic acid, 2.2% stearic acid, 14.5% oleic acid, 28.2% linolenic acid, and 3.1% linoleic acid. The emulsifier meets the specifications of the Food Chemical Codex (3rd ed.) (3a).

Animal Experiments

The animal experiments were approved by the Institutional Animal Care and Utilization Committee of the Case School of Medicine.

Preparation of rats. Male Sprague-Dawley rats were fed ad libitum with Harlan-Teklad rat chow. After an overnight fast, rats (235–317 g) were anaesthetized with intraperitoneal pentobarbital sodium (50 mg/kg dissolved in water). Catheters were inserted in a jugular vein and the ventral tail artery. Some rats were also fitted with a catheter in the duodenum (through a small laparotomy). After successful placement of catheters, saline (3.5 ml/h) and a maintenance dose of pentobarbital (5.8 mg/h) were infused into the jugular vein until the end of the experiments. A basal blood sample (70 μl) was obtained from the tail artery before the start of each protocol.

Protocol 1 (6 groups of 6–7 rats). Saline or a 10% triheptanoin emulsion was infused for 90 min either via the jugular vein at 0, 10, 20, 30, or 40% of caloric requirement or intraduodenally at 40% of caloric requirement. Arterial blood samples (70 μl) were taken at 60, 70, 80, and 90 min. At 91 min, 0.5 ml of blood was sampled from the abdominal aorta, after which the animal was killed by exsanguination.

Protocol 2 (4 groups of 7–9 rats). Rats were prepared as in protocol 1. They were infused for 120 min either with intravenous saline (group 1), intravenous triheptanoin (group 2, 40% of caloric requirement), or intraduodenal triheptanoin (groups 3 and 4, 40% of caloric requirement). In addition, they were infused intravenously with [6,6-2H2]glucose (1.2 μmol·min−1·kg−1) and with [U-13C3]glycerol (1.5 μmol·min−1·kg−1 in group 1, and 3.7 μmol·min−1·kg−1 in the triheptanoin groups 2, 3, and 4). In group 4, the tracers were infused intraduodenally with the triheptanoin emulsion. Arterial blood samples (70 μl) were taken at 0 (basal), 90, 100, 110, and 120 min. At 121 min, 0.5 ml of blood was again sampled from the abdominal aorta and the animals were killed as above.

Analytic procedures. All arterial blood samples (70 μl) were immediately pipetted into glass tubes containing a 1-ml aqueous solution of internal standards: [ω-1H]heptanoate (30 nmol), R,S-β-hydroxy-13CH3-butyrate (34 nmol), R,S-β-hydroxy-13CH3-pentanoate (28 nmol), and (for protocol 2) [U-13C3]glucose (50 nmol) and [3-13C]glycerol (2 or 5 nmol). After quick mixing, the solution of hemolyzed blood was treated with 40 μl of 1 M sodium borodeuteride in 0.1 M NaOH. The treatment with sodium borodeuteride converts unstable AcAc and BKP to the stable M1 BHB and M1 BHP, respectively, which can be distinguished by GC-MS from the unlabelled BHB and BHP present in blood (16). It also converts glucose to the M1 sorbitol. After 30 min, protein denaturation was achieved with 50 μl of saturated sulfosalicylic acid. In those experiments measuring glucose and glycerol, samples were divided prior to the addition of sulfosalicylic acid and HCl by transferring 0.2 ml of solution to a separate tube. The glucose and glycerol tubes were then acidified with HCl, whereas sulfosalicylic acid was added to the remaining tubes. Samples were stored at −20°C until assayed.

Assay of heptanoate and C2- and C3-ketone bodies. After treatment with sulfosalicylic acid, the slurry was acidified with HCl and extracted with 3 × 4 ml of diethyl ether. The combined extract was dried over Na2SO4, evaporated down to ~50 μl, and sonicated for 1 h with 3.5 μl of pentafluorobenzyl bromide, 10 μl of pyridine, and 37.5 μl of acetonitrile. After extraction with 3 ml of hexane, drying the extract over Na2SO4, and evaporating the solvent, the residue was reacted with 15 μl of of acetic anhydride and 30 μl of pyridine in 285 μl of ethyl acetate at room temperature overnight. This procedure converts 1 fatty acids to pentafluorobenzyl derivatives and 2 hydroxyacids to acetyl-pentafluorobenzyl derivatives. After extracting with diethyl ether, drying over Na2SO4 and evaporating with solvent, the residue was dissolved in 40 μl of ethyl acetate and 2 μl injected into an Agilent gas chromatograph linked to a 5973 MSD mass spectrometer. The chromatograph was equipped with a 30-m 77-5225 capillary column (Quadrex). The carrier gas was helium (30 ml/min), and the injection mode was splitless. The GC detector temperature was set at 190°C, and the transfer line was held at 280°C. The column temperature was increased from 100°C by 5°C/min to 235°C and held for 20 min. The mass spectrometer was operated under ammonia negative chemical ionization with the source pressure adjusted to obtain the maximal signal. The retention times and ions monitored were as follows: heptanoate (16 min, m/z 129, 132); BHP (20 min, m/z 145, 146, 151); BHP (21 min, m/z 159, 160, 164).

Assay of glucose and glycerol. After the addition of 1 drop of 12 N HCl, the solution was evaporated under nitrogen. The residue was reacted with 150 μl of acetic anhydride and 300 μl of pyridine, heated for 5 min at 100°C, and then left overnight at room temperature. The next day, samples were extracted with ethyl ether, and the extract was dried over Na2SO4 before evaporation. The residue was dissolved in 40 μl of ethyl acetate and either 2 μl (glucose assay) or 0.2 μl (glycerol assay) injected into the same chromatograph as above. The injection mode was either splitless (glucose) or split (glycerol). The column temperature was increased from 100°C by 20°C/min to 190°C, by 5°C/min from 190 to 220°C, and then by 20°C/min from 220 to 235°C, where it was held for 45 min. The mass spectrometer was operated under ammonia positive chemical ionization with the source pressure adjusted to obtain the maximal signal. The retention times and ions monitored were as follows: glycerol (8 min, m/z 237, 238, 239, 241), glucose converted to sorbitol (31 min, m/z 146, 151, 159), and glucose converted to sorbitol (31 min, m/z 453, 454, 455, 456, 459).

Data Analysis and Calculations

Total C3-ketone bodies were calculated as the sum of BHB plus AcAc, whereas total C2-ketone bodies were the sum of BHP plus BKP. Glucose and glycerol rates of appearance (Ra) were calculated (15) according to the steady-state equation:

\[ Ra = \frac{[IE_{infusate} - IE_{blood}]}{IE_{blood}} \times (INF) \]

where IEinfusate is the isotopic enrichment of the tracer infused, IEblood is the isotopic enrichment of blood, and INF is the rate of the tracer infusion (μmol·min−1·kg−1). To calculate endogenous glycerol Ra, from parenteral infusion of triheptanoin, glycerol Ra was
RESULTS

A major component of this study was the development of micro-analytical techniques so that all the assays required in the protocols could be conducted on 70 μl of whole blood. Pentfluorobenzoyl derivatives of carboxylic acids can be assayed using high-sensitivity ammonia negative chemical ionization mass spectrometry (11). Immediate treatment of the samples with sodium borodeuteride allowed stabilization of unstable AcAc and BKP by conversion to stable monodeuterated analytes. These monodeuterated analytes were distinguished by mass spectrometric analysis from the corresponding undeuterated analytes. As a result of the use of sodium borodeuteride during sample processing, glucose was assayed as sorbitol. All of the calibration curves were linear with substrate concentration (not shown). The assays were conducted on whole blood because orientation experiments revealed that the concentration of heptanoate in whole blood was 48–74% of the concentration in plasma. This probably results from the partial equilibration of heptanoate concentration between plasma and erythrocytes.

In the first study (protocol 1), we infused the triheptanoin emulsion for 90 min at 1) 0–40% of the caloric requirement intravenously or 2) 40% of the caloric requirement intraduodenally. Figure 1 shows that the intravenous infusion of triheptanoin (at 10–40% of caloric requirement) resulted in fairly stable blood heptanoate concentrations ranging from ~0.07 to 0.5 mM. In contrast, the intraduodenal infusion of triheptanoin at 40% of the caloric requirement resulted in a very low blood heptanoate concentration, i.e., ~0.02 mM. The blood heptanoate concentrations were undetectable (P < 0.005 mM) in basal samples and in samples from saline-infused rats. Because of the small sample of blood that we were able to obtain from each rat, blood concentrations of triheptanoin were not obtained.

The blood concentration of C5-ketone bodies (BKP + BHP; Fig. 2) was not detectable in basal samples or in the blood of saline-infused rats but increased with the rate of intravenous triheptanoin infusion. However, the highest concentration of C5-ketone bodies was achieved during the intraduodenal infusion at 40% of the caloric requirement (~0.22 mM; Fig. 2, top curve).

The basal blood concentrations of C3-ketone bodies (BHB + AcAc; Fig. 3) ranged from 0.7 to 1.1 mM. As a result of the anesthesia, the concentration of physiological C3-ketone bodies increased from the usual concentration of ~0.1 mM in conscious overnight-fasted rats to ~1 mM (25). The high basal concentration of C3-ketone bodies continued to increase significantly in saline-infused rats (P < 0.05), again presumably as a result of the prolonged fasting and anesthesia, but decreased significantly during the intravenous infusion of triheptanoin at 30 and 40% of the caloric requirement (P < 0.001).

In the second study (protocol 2), anesthetized rats were infused for 2 h with either saline iv, triheptanoin at 40% of the caloric requirement iv, or triheptanoin at 40% of the caloric requirement id. All rats were infused with [U-13C3]glycerol and [6,6-2H2]glucose. Tracer glycerol was used instead of a tracer fatty acid, because the measurement of lipolysis with tracer glycerol is less subject to analytic artifacts than the
The glycerol Ra (21.7 μmol·min⁻¹·kg⁻¹) the amount of glycerol infused intraduodenally as part of the triheptanoin emulsion. So, the endogenous Ra of glycerol of 25.4 μmol·min⁻¹·kg⁻¹ is a reasonable first approximation of peripheral lipolysis during the intraduodenal infusion of triheptanoin. A small correction to this Ra will be added below.

In the group of rats infused intraduodenally with triheptanoin and the glycerol tracer (Table 1, bottom row), the value of the total glycerol Ra (endogenous + exogenous) calculated from the rate of tracer infusion and the enrichment of glycerol in arterial blood (238 μmol·min⁻¹·kg⁻¹) is meaningless. This is because most of the labeled glycerol was taken up by the liver and could not mix with the glycerol derived from peripheral lipolysis. Indeed, the arterial enrichment of glycerol when [U-13C3]glycerol was infused intraduodenally was about one-fifth of the arterial enrichment of glycerol when the tracer was infused intravenously (compare rows 3 and 4 of Table 1). Thus, for the two experiments where triheptanoin was infused intraduodenally, taking into account the rates of tracer infusion and the arterial enrichments of glycerol, one can calculate the fraction of the intraduodenally infused glycerol tracer that escaped uptake by the liver. This fraction is equal to the ratio measurement with tracer fatty acids. Steady-state concentrations of both heptanoate and Cs-ketone bodies were virtually identical to those observed (Figs. 1 and 2) in the first set of experiments despite the 30-min longer infusion period of triheptanoin (not shown). The redox indicator ratio [BHP]/[BKP] stabilized at 1.5–2.0 after 90 min (not shown). Similar to the dose-response experiments, the greatest decrease in steady-state C4-ketone bodies occurred with intravenous infusion of triheptanoin (from 0.75 ± 0.07 down to 0.51 ± 0.05 mM, P < 0.001), whereas intraduodenal infusion of triheptanoin resulted in an attenuation of this decrease (from 1.05 ± 0.06 down to 0.89 ± 0.06 mM, P < 0.05).

The blood glucose concentration (Fig. 4A) was not significantly affected by the intravenous or intraduodenal infusion of triheptanoin except at 100 min, when glucose concentration was higher with intraduodenal triheptanoin infusion than with intravenous infusion (P < 0.05). However, the rate of glucose appearance (glucose Ra; Fig. 4B) was significantly increased by about one-half by the intraduodenal infusion of triheptanoin (P < 0.05). The basal blood glycerol concentrations in all four groups were elevated (0.42 ± 0.06 mM) compared with values from nonanesthetized animals (~0.15 mM (20)). This most likely resulted from the stress of the anesthesia and surgery, which stimulates lipolysis (5, 6). These effects are presumably mediated via increased concentrations of catecholamines and cortisol.

Table 1 shows the detail of the calculations of the Ra of glycerol in the four groups of rats of protocol 2. In saline-infused control rats, the glycerol Ra (21.7 μmol·min⁻¹·kg⁻¹) was similar to data from the literature (1). The intravenous infusion of triheptanoin tripled the arterial blood glycerol concentration (Fig. 4C) and about doubled the endogenous glycerol Ra (group 2; Table 1) compared with the saline control. In contrast, the intraduodenal infusion of triheptanoin did not affect the arterial blood glycerol concentration (Fig. 4C). This suggests that, during the intraduodenal infusion of triheptanoin, the glycerol derived from the intestinal hydrolysis of triheptanoin was cleared by the liver. Therefore, to calculate the endogenous glycerol Ra, one should not deduct from the total glycerol Ra (endogenous + exogenous, 25.4 μmol·min⁻¹·kg⁻¹) the amount of glycerol infused intraduodenally as part of the triheptanoin emulsion. So, the endogenous Ra of glycerol of 25.4 μmol·min⁻¹·kg⁻¹ is a reasonable first approximation of peripheral lipolysis during the intraduodenal infusion of triheptanoin. A small correction to this Ra will be added below.

![Fig. 3. Time course of blood concentrations of total C4-ketone bodies in the same experiments as Fig. 1.](image_url)

![Fig. 4. Time course of arterial blood glucose concentration (A), glucose rate of appearance (B), and glycerol concentration (C) in experiments of protocol 2 (groups 1–3). Saline (0%) or triheptanoin (40%) was infused from 0 to 120 min. Saline was infused parenterally, whereas 40% triheptanoin was infused iv or id. Data are presented as means ± SE (n = 7–9). The glucose concentration data of groups 3 and 4 were pooled. The glucose Ra data are shown only for groups 1–3 (iv infusion of labeled glucose).](image_url)
of enrichments of blood glycerol (2.3/12.6) normalized for equal rates of tracer glycerol infusion, i.e., (2.3/12.6) × (3.4/3.9) = 0.16. Thus 16% of the intraduodenally infused glycerol tracer escaped uptake by the liver. This percentage will now be used to calculate the peripheral \( R_g \) of glycerol in the two experiments where triheptanoin was infused intraduodenally.

First, in experiments with intraduodenal infusion of triheptanoin and intravenous infusion of tracer glycerol (group 3; Table 1), from the total glycerol \( R_g \) (25.4 \( \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \)) of enrichments of blood glycerol (2.3/12.6) normalized for equal rates of tracer glycerol infusion, i.e., (2.3/12.6) × (3.4/3.9) = 0.16. Thus 16% of the intraduodenally infused glycerol tracer escaped uptake by the liver. This percentage will now be used to calculate the peripheral \( R_g \) of glycerol in the two experiments where triheptanoin was infused intraduodenally.

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First, in experiments with intraduodenal infusion of triheptanoin and intravenous infusion of tracer glycerol (group 3; Table 1), the rate of glycerol tracer infusion that escaped liver uptake was 3.9 × 0.16 = 0.62 \( \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \). Therefore, the rate of peripheral lipolysis in these experiments was calculated using the classical steady-state equation:

\[
\text{endogenous } R_g, \text{ (group 3)} = 25.4 - (0.16 \times 26.1) = 21.2 \text{ \( \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \)}
\]

Second, in experiments with intraduodenal infusions of both triheptanoin and tracer glycerol (group 4; Table 1), the rate of glycerol tracer infusion that escaped liver uptake was 3.9 × 0.16 = 0.62 \( \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \). Therefore, the rate of peripheral lipolysis in these experiments was calculated using the classical steady-state equation:

\[
\text{endogenous } R_g, \text{ (group 4)} = [(1/(0.023) - 1)(0.62) = 26.3 \text{ \( \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \)}
\]

To summarize the data of Table 1, peripheral lipolysis (expressed as glycerol \( R_g \)) was not substantially affected by the intraduodenal infusion of triheptanoin (groups 3 and 4) but was about doubled by the intravenous infusion of triheptanoin (group 2).

DISCUSSION

The initial clinical trial of FOD patients with triheptanoin involved mixing the triglyceride with baby formula or with semisolid foodstuffs (22). However, emulsions of triheptanoin might be used in the future for the treatment of patients with long-chain FOD, either enterally or intravenously, for those who are going through acute decompensation. The latter patients are often comatose or confused and require treatment via intravenous or nasogastric administration. Temporary malabsorption syndromes such as gastroenteritis in long-chain FOD patients could also necessitate the use of intravenous administration. This is why we conducted this study on anesthetized rats receiving triheptanoin either intravenously or intraduodenally. A constant level of anesthesia and adequate hydration were imposed by infusing a low dose of sodium pentobarbital dissolved in saline.

Figures 1 and 2 show that, during the intraduodenal infusion of triheptanoin, virtually no heptanoate derived from triheptanoin hydrolysis escaped uptake by the liver. This is consistent with reports of essentially complete hepatic clearance of enterally administered medium-chain triglycerides (2, 9). It appears that a large fraction of the heptanoate reaching the liver is oxidized to BKP-CoA, which is partly converted to the C\(_4\)-ketone body BKP in the HMG-CoA cycle. BKP then equilibrates with BHP via a reaction catalyzed by BHB dehydrogenase. Indeed, standard solutions of unlabeled and labeled BHP and BKP are assayed with BHB dehydrogenase (16).

The total concentration of C\(_4\)- and C\(_5\)-ketone bodies (Figs. 2 and 3) did not exceed 1.2 mM in any group. Thus the intravenous infusion of triheptanoin at up to 40% of the caloric requirement did not induce a toxic ketosis.

The decrease in the concentration of C\(_4\)-ketone bodies during the intravenous infusion of triheptanoin at 30 or 40% of the caloric requirement was quite puzzling, especially in view of the reported increase in C\(_4\)-ketone bodies during infusion of even-chain triglycerides (medium- or long-chain) (3, 19, 23, 24). Because the heptanoate molecule has two acetyl moieties, these were expected to contribute somewhat to the production of C\(_4\)-ketone bodies. We considered the possibility that the intravenous infusion of triheptanoin could result in an inhibition of peripheral lipolysis, with a subsequent decrease in C\(_4\)-ketogenesis. We thus decided to assess the effect of triheptanoin on lipolysis in another series of experiments by measuring the \( R_g \) of glucose and glycerol. The glucose \( R_g \) was significantly increased by about one-half by the intraduodenal infusion of triheptanoin (\( P < 0.05; \) Fig. 4B). The increase in glucose \( R_g \) (\( \sim 10 \mu \)mol \( \cdot \)min\(^{-1} \cdot \)kg\(^{-1} \)) was only about one-third of the potential glucose equivalent of the triheptanoin infused (glycerol + propionyl-CoA moieties). This is because part of the propionyl-CoA moiety of heptanoate was converted to C\(_5\)-ketone bodies (Fig. 2). During the intravenous infusion of triheptanoin, the glucose \( R_g \) was not significantly increased (Fig. 4B), because a large fraction of the heptanoate derived from triheptanoin hydrolysis in blood was presumably taken up by nonglucagonemic peripheral tissues. In contrast, during the intraduodenal infusion, all the glycerol and heptanoate derived from triheptanoin hydrolysis in the gut reached the liver, the main glucagonemic organ.

The activation of lipolysis during the intravenous infusion of triheptanoin might result from the activation of lipoprotein lipase by the infused triglyceride. Karpe et al. (14) showed that intravenous infusion of long-chain triglycerides in humans

<table>
<thead>
<tr>
<th>Group</th>
<th>Substrate Infused</th>
<th>Route of Administration</th>
<th>Rate of Exogenous Glycerol Administration vs. Triheptanoin</th>
<th>Enrichment of Blood Glycerol (%)</th>
<th>Total Glycerol Ra (Endogenous + Exogenous)</th>
<th>Endogenous Glycerol Ra, kg/min <em>P</em> 0.05; ( \dagger P &lt; 0.01 ) vs. control group 1</th>
<th><em>P</em> 0.01 vs. control group 1</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline IV</td>
<td>IV</td>
<td>1.8 ± 0.1</td>
<td>9.0 ± 1.3</td>
<td>21.7 ± 1.8</td>
<td>21.7 ± 1.8</td>
<td>0.16</td>
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<tr>
<td>2</td>
<td>Triheptanoin IV</td>
<td>IV</td>
<td>3.8 ± 0.1</td>
<td>27.0 ± 0.5</td>
<td>53.0 ± 3.3</td>
<td>69.4 ± 4.8*</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>Triheptanoin ID</td>
<td>ID</td>
<td>3.4 ± 0.0</td>
<td>26.1 ± 0.2</td>
<td>12.6 ± 1.0†</td>
<td>25.4 ± 1.9</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>Triheptanoin ID</td>
<td>ID</td>
<td>3.9 ± 0.2</td>
<td>28.2 ± 1.0</td>
<td>2.3 ± 0.6†</td>
<td>[238 ± 31]</td>
<td>0.01</td>
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</table>
leads to an up to ninefold activation of lipoprotein lipase in arterialized blood. Nordenstrom et al. (18) demonstrated a similar increase when a combination medium-chain/long-chain triglyceride infusion was infused, with greater lipoprotein lipase activities noted with the medium-chain/long-chain infusion than with long-chain infusion alone. In this latter set of experiments, there was a significant correlation (r = 0.77) between plasma triglyceride concentrations and lipoprotein lipase activity during triglyceride infusion.

In our experiments where triheptanoin was infused intraduodenally (groups 3 and 4; Table 1), there was no increase in endogenous glycerol Ra, presumably because triglyceride particles did not enter the vascular bed and thus did not activate lipoprotein lipase.

During the intravenous infusion of triheptanoin, the activation of lipolysis presumably led to the release of long-chain fatty acids from adipose tissue. However, the arterial concentration of C4-ketone bodies decreased. This is an apparent contradiction, as an increase in the supply of long-chain fatty acids to the liver would be expected to result in enhanced C4-ketogenesis. We suggest that the bulk of the long-chain fatty acids released by lipolysis was reesterified, whereas heptanoate was preferentially oxidized. Preferential oxidation of heptanoate would be favored because 1) it does not accumulate in adipose tissue (26) and 2) its oxidation bypasses the carnitine palmitoyltransferase system. Reesterification of long-chain fatty acids occurs partly in the liver, where the glycerol moiety of triheptanoin is activated to α-glycerophosphate, an intermediate of triglyceride synthesis. However, there are other reesterification sites (13), in particular, muscle, which uses plasma glycerol for triglyceride synthesis (10) and which is a site of triglyceride storage (8, 12). Reesterification of long-chain fatty acids in muscle was probably promoted by 1) the tripling in glycerol concentration during the intravenous infusion of triheptanoin (Fig. 4C) and 2) the supply of energy substrates in the form of heptanoate and C4-ketone bodies. The increase in glucose Ra and the slight hyperglycemia induced by triheptanoin infusion may have led to the release of insulin, which promoted reesterification of long-chain fatty acids in muscle and adipose tissue. Because fatty acids shorter than nine carbons are not stored as glycerides in the rat (26), the heptanoate moiety of triheptanoin was presumably oxidized in preference to the long-chain fatty acids released by enhanced lipolysis. Experiments in dogs fitted with duodenal, transhepatic, and transmuscle catheters are planned to test the above-mentioned mechanisms.

The data of this translational physiology study have a number of clinical implications for the potential use of triheptanoin emulsion in the treatment of long-chain FOD decompensation. Currently, only the enteral form of triheptanoin is available for some clinical conditions, one might prefer to administer triheptanoin intraduodenally to favor supplying C4-ketone bodies over heptanoate to peripheral tissues. It is likely that C4-ketone bodies, like C2-ketone bodies, can be taken up and used by the brain, which has very high activities of 3-oxoacid-CoA transferase and BHB dehydrogenase (7). The intravenous administration of triheptanoin leads to an activation of lipolysis, which would not be desirable during the acute treatment of long-chain FOD decompensation. However, this stimulation of lipolysis could then be countered by the reesterification of long-chain fatty acids. Reesterification would be enhanced by the infusions of glucose and insulin that are used in severe cases of FOD decompensation, so the addition of parenteral triheptanoin to the current treatment by glucose and insulin may be beneficial to these patients. In mild cases of FOD decompensation, enteral administration of triheptanoin emulsion would be preferred, in the absence of diarrhea.

We want to stress that we do not imply that this study, conducted in anesthetized rats, clears the way for the utilization of triheptanoin emulsion for the treatment of decompensated patients with long-chain FOD. This will require additional studies in larger animals with metabolic rates per kilogram closer to those of humans. Although heptanoate does not accumulate in tissue glycercides (26), detailed studies on the influence of triheptanoin infusion on the profile and kinetics of plasma lipids, including the concentration of plasma triheptanoin, will need to be conducted in various species, including in genetically engineered mice with FOD.

GRANTS

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REFERENCES


