Parenteral and Enteral Metabolism of Anaplerotic Triheptanoin in Normal Rats

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Footnote

1. The improvement of the neurological status of a child with pyruvate carboxylase deficiency during treatment with triheptanoin (17) suggests that C₅-ketone bodies are taken up and used by the brain.
ABSTRACT

A new chronic treatment for inherited disorders of long-chain fatty acid oxidation involves administering up to one-third of dietary calories as triheptanoin, a medium-odd-chain triglyceride (Roe et al J. Clin. Invest. 110: 259, 2002). Heptanoate and C₅-ketone bodies derived from its partial oxidation in liver are precursors of anaplerotic propionyl-CoA in peripheral tissues. It was hypothesized that increasing anaplerosis in peripheral tissues would boost energy production. In the present study, we tested the potential of a triheptanoin emulsion as an intravenous nutrient. Normal rats were infused with triheptanoin intravenously or intraduodenally at up to 40% of caloric requirement. The blood concentration ratio (heptanoate)/(C₅-ketone bodies) was high with intravenous, and low with intraduodenal triheptanoin infusion. During intravenous infusion of triheptanoin, lipolysis was stimulated, but appeared compensated by fatty acid re-esterification. During intraduodenal infusion of triheptanoin, lipolysis was not stimulated. Our data support the hypothesis that intravenous triheptanoin could be used to treat decompensated patients with long-chain fatty acid oxidation disorders.
INTRODUCTION

Inherited fatty acid oxidation disorders (FOD) include defects of the cell membrane carnitine transporter, the “carnitine cycle” (CPT I, translocase, CPT II), or the mitochondrial β-oxidation spiral. The most common disorders of β-oxidation affect very-long chain acyl-CoA dehydrogenase, mitochondrial trifunctional protein, isolated long-chain hydroxyacyl-CoA dehydrogenase, medium-chain- and short-chain acyl-CoA dehydrogenase. Patients with long-chain FOD present commonly with recurrent hypoketotic hypoglycemia, hypertrophic or dilated cardiomyopathy, cardiac arrhythmias, rhabdomyolysis, muscle weakness, and hypotonia, (for a review, see (21)). There is considerable phenotypic variation associated with nearly all of these disorders.

The classical chronic treatment of long-chain FOD involves frequent feeding with a diet adjusted so as to lower long-chain fat intake from the usual 30-35% of total Kcal to about 20%, including essential fatty acids. The decrease in energy from long-chain fats is partly compensated by an increase in carbohydrates, often with cornstarch at bed time. In addition, even-carbon medium chain triglycerides (trioctanoin/tridecanoin) are added to the diet. This is because fatty acids with 8-10 carbons enter the mitochondrion as carboxylates which, after activation, require only those β-oxidative enzymes with medium- and short-chain length specificity. The dietary treatment with medium-chain triglycerides is obviously restricted to long-chain disorders and is contraindicated for medium- and short-chain deficiencies.

A new strategy was recently conceived for the dietary treatment of long-chain FOD, i.e., 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, C.R., unpublished). Also, treatment with triheptanoin improved for six months the condition of a patient with almost complete deficiency in 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 (BKP) and β-hydroxypentanoate (BHP) via the HMG-CoA cycle. The C₅-ketone bodies are analogs of 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 D-β-hydroxybutyrate dehydrogenase, 3-oxoacid-CoA transferase, and acetoacetyl-CoA thiolase. Thus, after administration of heptanoate, propionyl-CoA and acetyl-CoA are formed in peripheral tissues by (i) β-oxidation of heptanoate, and (ii) 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 which might be used in the future for the treatment of acute long-chain FOD decompensation, via either intravenous or enteral infusion. An emulsion of triheptanoin was infused intravenously or intraduodenally, 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.
METHODS

Materials: [U-13C6]glucose, [6,6-2H2]glucose, [2H8]glycerol, [U-13C3]glycerol, [ω-2H3]heptanoic acid, 2H2O, NaO2H, sodium borodeuteride (NaB2H4) and general chemicals were purchased from Isotec/Sigma-Aldrich. Pentafluorobenzyl bromide was purchased from Pierce. An internal standard of R,S-β-hydroxy-[2H5]pentanoate was prepared by incubating ethyl β-ketopentanoate in 2H2O + NaO2H, followed by reducing β-keto-[2H4]pentanoate with sodium borodeuteride (16). A 10% sterile emulsion of triheptanoin was kindly donated by Sasol Germany GmbH. 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% triester, with the remaining 2.5% being monoester and diester. The emulsion was stabilized with 1% of Imwitor 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% linoleic acid, and 3.1% linolenic acid. The emulsifier meets the specifications of the Food Chemical Codex (3rd Edition 1981, pp 98-99).

Animal experiments:

The animal experiments have been 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 (235-317g) were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg dissolved in water). Catheters were inserted in a jugular vein and in the ventral tail artery. Some rats were also fitted with a catheter in the duodenum (through a
small laparotomy). Following successful placement of catheters, saline (3.5 ml/hr) and a maintenance dose of pentobarbital (5.8 mg/hr) 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 sacrificed 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 IV saline (group 1), IV 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 2,3,4). 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 sacrificed as above.

Analytical procedures: All arterial blood samples (70 µL) were immediately pipetted into glass tubes containing a 1 ml aqueous solution of internal standards: [ω-²H₃]heptanoate (30 nmol), R,S-β-hydroxy-[²H₆]butyrate (34 nmol), R,S-β-hydroxy-[²H₅]pentanoate (28 nmol), and (for Protocol 2): [U-¹³C₆]glucose (50 nmol) and [²H₅]glycerol (2 or 5 nmol). After quick mixing, the solution of hemolysed 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 unlabeled 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, while sulfosalicylic acid was added to the remaining tubes. Samples were stored at -20°C until assayed.

Assay of heptanoate, C₄- and C₅-ketone bodies: After treatment with sulfosalicylic acid, the slurry was acidified with HCl and extracted with 3 x 4 ml of diethyl ether. The combined extract was dried over Na₂SO₄, evaporated down to about 50 µL, and sonicated for 1 hour with 3.5 µL pentafluorobenzyl bromide, 10 µL pyridine, and 37.5 µl acetonitrile. After extraction with 3 ml hexane, drying the extract over Na₂SO₄, and evaporating the solvent, the residue was reacted with 15 µL of acetic anhydride, 30 µL of pyridine, and 285 µl of ethyl acetate at room temperature overnight. This procedure converts (i) fatty acids to pentafluorobenzyl derivatives, and (ii) 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-OV-225 capillary column (Quadrex Corporation). The carrier gas was helium (30 ml/min) and the injection mode was splitless. The GC injector temperature was set at 190°C, and the transfer line was held at 280°C. The column temperature was increased from 100° 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); BHB (20 min, m/z 145, 146, 151); BHP (21 min, m/z 159, 160, 164).

**Assay of glucose and glycerol:** Following 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 100ºC, then left overnight at room temperature. The next day, samples were extracted with ethyl ether and the extract 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 100ºC by 20ºC/min to 190ºC, by 5 C/min from 190ºC to 220ºC, then by 20ºC/min from 220ºC to 235ºC, 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 236, 237, 238, 239, 241), glucose converted to sorbitol (31 min, m/z 453, 454, 455, 456, 459).

**Data Analysis and Calculations:** Total C₄ ketone bodies were calculated as the sum of BHB and AcAc, while total C₅ ketone bodies were the sum of BHP and BKP. Glucose and glycerol Ra were calculated (15) according to the steady-state equation:

$$R_a = \left(\frac{[IE_{infusate}]}{IE_{blood}}-1\right)\times(INF),$$

where IE_{infusate} is the isotopic enrichment of the tracer infusate, IE_{blood} 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 corrected for the total amount of free glycerol present in the infusate using the equation
Endogenous glycerol \( R_a = \frac{\left( \frac{I_{E_{\text{infusate}}}}{I_{E_{\text{blood}}}} - 1 \right) \cdot I_{\text{NF}}}{G_{\text{inf}}} \)

where \( I_{E_{\text{infusate}}} \) and \( I_{E_{\text{blood}}} \) are the isotopic enrichments of the tracer in infusate and blood, respectively, \( I_{\text{NF}} \) is the infusion rate of the tracer (\( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \)), and \( G_{\text{inf}} \) is the total glycerol infusion rate (\( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \)) determined by measurement of the total glycerol concentration in the triheptanoin emulsion after alkaline hydrolysis. Additional calculations were conducted to compute the isotopic data from experiments where triheptanoin was infused in the duodenum of rats (see text). All statistics (ANOVA with Tukey’s post hoc analysis and t-tests) were performed using GB-Stat statistical software on a personal computer. Data are reported as means \( \pm \) SEM. \( P \leq 0.05 \) was considered significant.
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. Pentafluorobenzyl 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 BHB and BHP (4; 16). 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 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 to 74% of the concentration in plasma. This probably results from the partial equilibration of heptanoate concentration between plasma and erythrocytes.

In a first study (Protocol 1), we infused the triheptanoin emulsion for 90 min (i) at 0 to 40% of the caloric requirement intravenously, or (ii) at 40% of the caloric requirement intraduodenally. Fig 1 shows that the intravenous infusion of triheptanoin (at 10 to 40% of the caloric requirement) resulted in fairly stable blood heptanoate concentrations ranging from about 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., about 0.02 mM. The blood heptanoate concentrations were undetectable (< 0.005 mM) in basal samples and in samples from saline-infused rats. Due to 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 (about 0.22 mM; Fig 2, upper curve).

The basal blood concentrations of C₄-ketone bodies (BHB + AcAc, Fig 3) ranged from 0.7 to 1.1 mM. As a result of the anesthesia, the concentration of physiological C₄-ketone bodies increases from the usual concentration of about 0.1 mM in conscious overnight-fasted rats to about 1 mM (25). The high basal concentration of C₄-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 hr with either saline intravenously, triheptanoin at 40% of the caloric requirement intravenously, or triheptanoin at 40% of the caloric requirement intraduodenally. 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 analytical artifacts than the measurement with tracer fatty acids. Steady state concentrations of both heptanoate and C₅ ketone bodies were virtually identical to those observed (Figs 1, 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), while
duodenal 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 R_a, 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 to values from non-anesthetized animals (about 0.15 mM (20)). This most likely results 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 R_a of glycerol in the four groups of rats of Protocol 2. In saline infused controls rats, the glycerol R_a (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 R_a (Table 1, group 2), compared to 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 R_a, one should not deduct from the total glycerol R_a (endogenous + exogenous, 25.4 µmol•min^{-1}•kg^{-1}) the amount of glycerol infused intraduodenally as part of the triheptanoin emulsion. So, the endogenous R_a 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 $R_a$ will be added below.

In the group of rats infused intraduodenally with triheptanoin and the glycerol tracer (Table 1, last row), the value of the total glycerol $R_a$ (endogenous + exogenous) calculated from the rate of tracer infusion and the enrichment of glycerol in arterial blood ($238 \, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) 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 $[^2\text{H}_5]\text{glycerol}$ 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 which escaped uptake by the liver. This fraction is equal to the ratio of enrichments of blood glycerol ($2.3/12.6$) normalized for equal rates of tracer glycerol infusion, i.e., $(2.3/12.6)\times(3.4/3.9) = 0.16$. Thus 16% of the intraduodenally infused glycerol tracer escaped uptake by the liver. This percentage will now be used to calculate the peripheral $R_a$ 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 (Table 1, group 3), from the total glycerol $R_a$ ($25.4 \, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$, i.e., endogenous + exogenous) one deducts 16% of the rate of glycerol administration via triheptanoin:

$$\text{Endogenous } R_a \text{ (group 3)} = 25.4 - (0.16 \times 26.1) = 21.2 \, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$$
Second, in experiments with intraduodenal infusions of both triheptanoin and tracer glycerol (Table 1, group 4), the rate of glycerol tracer infusion which escaped liver uptake was $3.9 \times 0.16 = 0.62 \, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$. Therefore, the rate of peripheral lipolysis in these experiments was calculated using the classical steady state equation:

$$\text{Endogenous glycerol } R_a \text{ (group 4)} = \left(\frac{1}{0.023} - 1\right)(0.62) = 26.3 \, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}.$$ 

To summarize the data of Table 1, peripheral lipolysis (expressed as Glycerol $R_a$) 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 semi-solid 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/and naso-gastric administration. Temporary mal-absorption 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.

Figs 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 C₅-ketone body BKP in the hydroxymethylglutaryl-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 C₄- and C₅-ketone bodies (Figs 2, 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 C₄-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 C₄-ketone bodies during infusion of even-chain triglycerides (medium- or long-chain) (3; 19; 23; 24). Since the heptanoate molecule has two acetyl moieties, these were expected to contribute somewhat to the production of C₄-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 C₄-ketogenesis. We thus decided to assess the effect of triheptanoin on lipolysis in another series of experiments by measuring the rates of appearance (Ra) of glucose and glycerol. The glucose Ra was significantly increased by about one half by the intraduodenal infusion of triheptanoin (Fig 4B, p < 0.05). The increase in glucose Ra (about 10 µmol•min⁻¹•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 C₅-ketone bodies (Fig 2). During the intravenous infusion of triheptanoin, the glucose Ra was not significantly increased (Fig 4B) because a large fraction of the heptanoate derived from triheptanoin hydrolysis in blood was presumably taken up by non-gluconeogenic 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 gluconeogenic 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. showed that intravenous infusion of long-chain triglycerides in humans leads to an up to nine-fold activation of lipoprotein lipase in arterialized blood (14). Nordenstrom et al. 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 (18). 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 (Table 1, groups 3, 4),
there was no increase in endogenous glycerol $R_a$. This is 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 $C_4$-
ketone bodies decreased. This is an apparent contradiction since an increase in the supply of
long-chain fatty acids to the liver would be expected to result in enhanced $C_4$-ketogenesis. We
suggest that the bulk of the long-chain fatty acids released by lipolysis was re-esterified while
heptanoate was preferentially oxidized. Preferential oxidation of heptanoate would be favored
because (i) it does not accumulate in adipose tissue (26), and (ii) its oxidation bypasses the
carnitine palmitoyltransferase system. Re-esterification of long-chain fatty acids occurs partly
in the liver where the glycerol moiety of triheptanoin is activated to $\alpha$-glycerophosphate, an
intermediate of triglyceride synthesis. However, there are other sites of re-esterification (13), in
particular muscle which uses plasma glycerol for triglyceride synthesis (10), and which is a site
of triglyceride storage (8; 12). Re-esterification of long-chain fatty acids in muscle was probably
promoted by (i) the tripling in glycerol concentration during the intravenous infusion of
triheptanoin (Fig 4C), and (ii) the supply of energy substrates in the form of heptanoate and $C_5$-
ketone bodies. The increase in glucose $R_a$ and the slight hyperglycemia induced by
triheptanoin infusion may have led to the release of insulin which promoted re-esterification of
long-chain fatty acids in muscle and adipose tissue. Since fatty acids shorter than 9 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, trans-hepatic and trans-muscle catheters are planned to test the above 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 therapy, as part of semi-solid meals or baby formula. However, parenteral triheptanoin has the potential to be used during an acute decompensation or during episodes of malabsorption. The production of propionyl-CoA from heptanoate or/and C\textsubscript{5}-ketone bodies would replenish the pool of Citric acid cycle intermediates and restore energy production. Since the heart is the organ with the highest activity of 3-oxoacid-CoA transferase \textsuperscript{(7)}, it forms propionyl-CoA from both heptanoate and C\textsubscript{5}-ketone bodies.

The data of Figs 1 and 2 show that the intravenous and intraduodenal infusions of triheptanoin lead to opposite blood concentration ratios of (heptanoate)/(C\textsubscript{5}-ketone bodies). In some clinical conditions, one might prefer to administer triheptanoin intraduodenally to favor supplying C\textsubscript{5}-ketone bodies over heptanoate to peripheral tissues. It is likely that C\textsubscript{5}-ketone bodies, like C\textsubscript{4}-ketone bodies, can be taken up and used by the brain\textsuperscript{1} which has very high activities of 3-oxoacid-CoA transferase and \(\beta\)-hydroxybutyrate dehydrogenase \textsuperscript{(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 re-esterification of long-chain fatty acids. Re-esterification would be enhanced by the infusions of glucose and insulin which 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 Kg closer to those of humans. Although heptanoate does not accumulate in tissue glycerides (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.
Reference List


16. **Leclerc J, Des Rosiers C, Montgomery JA, Brunet J, Ste-Marie L, Reider MW, Fernandez CA, Powers L, David F and Brunengraber H.** Metabolism of R-beta-


Table 1

Calculations of Glycerol $R_a$ in Protocol 2

Triheptanoin was infused at 40% of caloric requirement either intravenously (IV) or intraduodenally (ID). All rates are expressed in µmol·min$^{-1}$·kg$^{-1}$ (mean ± SE, n = 7 - 9). Rates shown in italic characters (last column) result from non-standard computations described in the text. The rate shown between brackets in the bottom row is not valid (see text). The enrichment of glycerol is expressed in mol percent.

<table>
<thead>
<tr>
<th>Group</th>
<th>Substrate Infused &amp; Route of Administration</th>
<th>Route of Tracer Glycerol Administration</th>
<th>Rate of Tracer Glycerol Administration</th>
<th>Rate of Exogenous Glycerol Administration via Triheptanoin</th>
<th>Enrichment of Blood Glycerol (%)</th>
<th>Total Glycerol $R_a$ (Endogenous + Exogenous)</th>
<th>Endogenous Glycerol $R_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline IV</td>
<td>IV</td>
<td>1.8 ± 0.1</td>
<td>N/A</td>
<td>9.0 ± 1.3</td>
<td>21.7 ± 1.8</td>
<td>21.7 ± 1.8</td>
</tr>
<tr>
<td>2</td>
<td>Triheptanoin IV</td>
<td>IV</td>
<td>3.8 ± 0.1</td>
<td>27.0 ± 0.5</td>
<td>5.3 ± 0.3**</td>
<td>69.4 ± 4.8*</td>
<td>42.4 ± 4.5*</td>
</tr>
<tr>
<td>3</td>
<td>Triheptanoin ID</td>
<td>IV</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>21.2</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>26.3</td>
</tr>
</tbody>
</table>

*: p < 0.01 compared to control group 1.
**: p < 0.01 compared to control group 1, after normalization of rates of tracer glycerol infusion.
FIGURE LEGENDS

**Fig 1.** Time course of blood heptanoate concentration during infusion of saline (0%) or triheptanoin (at 10%, 20%, 30%, or 40% of caloric requirement) which was infused from 0 to 90 min. All infusions were intravenous (IV) except for one group which received triheptanoin intraduodenally (ID) at 40% of caloric requirement. Data are reported as mean ± SEM for all groups.

**Fig 2.** Time course of blood concentrations of total C₅-ketone bodies in the same experiments as Fig 1.

**Fig 3.** Time course of blood concentrations of total C₄-ketone bodies in the same experiments as Fig 1.

**Fig 4.** Time course of arterial blood glucose concentration (panel A), glucose $R_a$ (panel B) and glycerol concentration (panel 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, while 40% triheptanoin was infused either parenterally (IV) or intraduodenally (ID). Data are presented as mean ± SEM ($n = 7$ to 9). The glucose concentration data of groups 3 and 4 were pooled. The glucose $R_a$ data are shown only for groups 1-3 (intravenous infusion of labeled glucose).
Fig 4

A

Glucose (mM)

40% ID
Saline
40% IV

B

Glucose \( R_2 \) (μmol·kg⁻¹·min⁻¹)

40% ID
Saline
40% IV

C

Glycerol (mM)

0 90 100 110 120

0.0 0.5 1.0 1.5 2.0

0 90 100 110 120

0.0 0.5 1.0 1.5 2.0

0 90 100 110 120

0.0 0.5 1.0 1.5 2.0