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No effect of triheptanoin in patients with phosphofructokinase deficiency

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Abstract

Phosphofructokinase deficiency (PFKD) is a rare disorder of glycogen metabolism. The lack of phosphofructokinase activity blocks the oxidative pathway from glucose and glycogen to pyruvate. Patients suffer from myopathy, exercise intolerance, and myoglobinuria. Currently, there is no specific treatment for PFKD. We hypothesized that 2 weeks treatment with triheptanoin could improve oxidative metabolism during exercise by bypassing the blocked pyruvate generation in PFKD. The study was a randomized, double-blind, placebo-controlled crossover study. Three genetically verified patients completed two treatment periods of 14 days each with triheptanoin ($0.3\text{--}1\text{ g} \times \text{kg}^{-1} \times \text{day}^{-1}$) or placebo liquid. Primary outcomes were heart rate, fatty acid and total oxidation measured via stable isotope and indirect calorimetry methodology during submaximal exercise. Triheptanoin did not improve the primary outcome heart rate during submaximal exercise compared to placebo. Palmitate oxidation was increased during submaximal exercise in one patient but did not increase in the two other patients during triheptanoin treatment. Palmitate production and palmitate utilization increased during exercise and increased to a greater extent with triheptanoin treatment in all three patients. This study suggests that triheptanoin treatment has no effect on heart rate or exercise performance despite increased palmitate production and utilization in patients with PFKD.

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1. Introduction

Phosphofructokinase deficiency (PFKD) is a rare disorder of glycogen metabolism. This deficiency is known as glycogen storage disease VII (GSD VII) [1]. The disease is autosomal recessively inherited, originally described in a Japanese family [1]. Onset of PFKD typically occurs during childhood. Patients usually present with myopathy, exercise

intolerance, and myoglobinuria, but four different clinical presentations have been described: (a) a classical form, with symptoms as above (b) a severe infantile form, with hypotonia, progressive myopathy, cardiopathy, and respiratory failure, (c) a late onset form, with proximal myopathy, typically arising in the fifth decade and (d) a hemolytic form, with characteristic non-spherocytic hemolytic anemia without muscle involvement [2–4].

The deficient enzyme, phosphofructo-1-kinase (PFK), is the rate-limiting enzyme in the glycolytic pathway. In human skeletal muscle, it is expressed through the *PFKM* gene [5]. The lack of PFK activity blocks the oxidative pathway from glucose and glycogen to pyruvate. As a consequence,

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glucose cannot be utilized in muscle energy metabolism. In fact, increase in blood glucose decreases exercise tolerance in patients with PFKD, due to inhibition of lipolysis leading to a reduction of energy from the alternative oxidative substrates, free fatty acids, and ketone bodies [6].

Metabolic studies of other glycogenosis - McArdle disease and Debrancher deficiency - have shown an energy deficit on muscle contraction, caused by reduced skeletal muscle oxidation of carbohydrates, which is only partially mitigated by a compensatory increase in fatty acid oxidation (FAO). Despite increasing availability of free fatty acids (FFA) during exercise, FAO is not increased proportional to the availability of FFA, even though the energy deficit is maintained [7,8]. This may be due to impaired anaplerosis of the tricarboxylic acid (TCA) cycle, as no pyruvate is generated from the blocked glycogenolysis, thus limiting oxaloacetate production, which is a key intermediate of the TCA cycle. The reduced supply of oxaloacetate slows the TCA cycle. As a consequence, less acetyl-CoA is metabolized, restricting fat oxidation and ATP production, which is key for exercise tolerance in patients with PFKD.

Triheptanoin derivatives may function as an alternative source of anaplerotic substrates for the TCA cycle, bypassing the blocked pyruvate generation in PFKD. The liquid has been used as treatment for several other inherited metabolic diseases characterized by either impaired supply of TCA cycle intermediates or fatty acid degradation [9].

Triheptanoin contains glycerol and three 7-carbon fatty acids – heptanoate (C7). In the mitochondria, the odd-chained heptanoate undergoes β -oxidation resulting in two molecules of acetyl-CoA and one molecule of propionyl CoA. Propionyl-CoA will be further converted to the anaplerotic substrate, succinyl-CoA, refueling the TCA cycle, thus compensating for the lack of oxaloacetate from pyruvate [10]. Additionally, the liver breaks down C7 to C5-ketone bodies, which are later metabolized in muscle and other tissues to succinyl-CoA. [11].

In this study, we hypothesized that treatment with triheptanoin liquid could improve fatty acid metabolism by fueling the TCA cycle with substrates, compensating for the lack of pyruvate-generated oxaloacetate and thereby enhance oxidative metabolism during exercise.

2. Methods & materials

We conducted a randomized, double-blind crossover study on the effect of triheptanoin in patients with PFKD. Participants completed a treatment period of ≥ 14 days followed by $a \geq 7$ -day washout period before a second ≥ 14 days treatment period. Participants were randomized 1:1 to a treatment sequence of either placebo or active treatment in the first treatment period followed by the alternate treatment in the second period. Participants arrived at the clinic for one screening visit followed by four assessment visits, one before and one on the last day of each treatment periods.

2.1. Participants

Inclusion criteria included (a) a genetically and/or biochemically verified diagnosis of PFKD, and (b) age ≥ 15 years. Women of fertile age had to be on contraceptive treatment.

Exclusion criteria included (a) significant cardiac or pulmonary disease, (b) pregnancy or breastfeeding, (c) treatment with beta-blockers, (d) inability to perform cycling exercise, (e) risk of musculoskeletal injury, or (f) any other significant disorder that might confound the interpretation of the findings.

The study was conducted at the Copenhagen Neuromuscular Center research facility, Rigshospitalet, Copenhagen, Denmark. Participants were recruited from the Consultation Neuromusculaire, Hospital Raymond-Poincare, Garches, France, and the MRC centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, London, England. All participants were recruited at their regular visits to these clinics. Further, participants were recruited online through a patient interest Facebook group.

2.2. Study treatment

Triheptanoin is an odorless and tasteless liquid. Placebo treatment was safflower liquid with similar characteristics to triheptanoin without odd-chained heptanoate. Both treatments were delivered by Ultragenyx Pharmaceutical Inc. (Novato, CA) and came in 1L round, amber-colored glass bottles (USP, pH. Eur. Grade). During the first 7 days of treatment, participants were treated with an increasing dose of 0.3, 0.5 and $0.7 \text{ g} \times \text{kg}^{-1} \times \text{day}^{-1}$ followed by 7 days on full dose ($1 \text{ g} \times \text{kg}^{-1} \times \text{day}^{-1}$). The full dose aimed at providing 30–35% of total calories intake and substituting ingestion of other types of fat. Dosages were divided and ingested with 3–4 meals daily, while the participants were on an isocaloric diet of fat and sugar intake restriction. They received systematic dietary instructions. The dosage distributions were planned with investigators at the two baseline visits. Participants received the bottles with treatment along with a dietary guideline, dosing tubes, and a treatment diary to register deviations or comments. Prior to the exercise test, participants were instructed to avoid corn-containing dietary products as to reduce interference between naturally occurring isotopes and the infused isotopes during tests.

2.3. Blinding and randomization

An un-blinded pharmacist at Regions Apoteket, Rigshospitalet, Copenhagen, Denmark created a randomization list of 200 unique 4-digit numbers between 1000 and 1199 and kept track of which bottle numbers were used. An un-blinded representative at Ultragenyx Pharmaceutical Inc. assigned each number to a treatment bottle. Ultragenyx Pharmaceutical Inc. organized the labeling and shipped a bulk of treatment to the study side upon

request. At inclusion of new participants, the un-blinded pharmacist assigned bottles to the participant with treatment sequences 1:1. The investigator was informed by e-mail which bottles to hand out in the two treatment periods.

At the end of the study, the un-blinded pharmacist shared the treatment assignment list with the investigators. Participants were informed of their individual treatment sequences.

2.4. Outcome measures

Primary outcome measures were assessed during constant load exercise and included (a) heart rate (HR) and (b) fatty acid oxidation measured via stable isotope technique and indirect calorimetry.

Secondary outcome measures included (a) glucose rate of appearance (R_a) and disappearance (R_d), (b) maximal workload capacity (W_{max}) and maximal oxidative capacity (VO_{2max}), (c) rate of perceived exertion (Borg score) during constant workload cycling (RPE_{const}), (d) changes in plasma concentrations of lactate, ammonia, glucose, free fatty acids (FFA), heptanoate, and hormones, (e) self-rated daily function scores on a modified short form health questionnaire (SF-36) and Bouchards energy expenditure questionnaire.

2.5. Maximal exercise test

After inclusion, participants performed a maximal exercise test on a seated ergometer cycle (Corival recumbent, Lode, Groningen, The Netherlands) to assess the participants' exercise capacity and to estimate a workload suitable for the exercise test on submaximal test days 1–4. Breath-by-breath exchange rate of O_2 (VO_2) and CO_2 (VCO_2) were measured with a metabolic cart (CPET, Cosmed, Rome, Italy).

2.6. Submaximal test days 1–4

Participants arrived at the exercise laboratory in the morning having been fasted overnight and were served a standardized breakfast (described below). After breakfast, a peripheral venous catheter was inserted in each arm for stable isotope tracer infusion and for blood sampling, respectively. A heating pad covered the arm to arterialize the venous blood sampling.

Two hours prior to exercise, a single priming dose of $Na^{13}HCO_3$ ($0.0735 \text{ mg} \times \text{kg}^{-1}$) and $6,6\text{-}^2H_2\text{-glucose}$ ($2.44 \text{ mg} \times \text{kg}^{-1}$) dissolved in saline was delivered intravenously, followed by a constant-rate infusion of $U\text{-}^{13}C16\text{-palmitate}$ ($0.02 \text{ } \mu\text{mol} \times \text{kg}^{-1} \times \text{min}^{-1}$) and $6,6\text{-}^2H_2\text{-glucose}$ ($0.7 \text{ } \mu\text{mol} \times \text{kg}^{-1} \times \text{min}^{-1}$). Infusions were delivered by a Gemini PC2 pump (IMED, San Diego, California). Tracer infusions were prepared as previously described [7]. The infusion rates were doubled at the onset of exercise.

Participants performed a submaximal exercise test of 30 min at an intensity that matched 65% of the participant's VO_{2max} followed by an increasing load every minute until maximal exercise. Heart rate and score of perceived exertion

(Borg score) were noted every second minute for 30 min and every minute afterwards. Blood and breath samples were collected 20 min before exercise start and then every 10 min until exercise end. Additional samples were taken at 5 min after start and immediately after maximal exercise. In between sampling, VO_2 and VCO_2 were measured using indirect calorimetry.

2.7. Standardized breakfast

Participants were served a meal of low fat, sugar-free yogurt (Cheasy 1% Arla Foods amba, Viby, Denmark, $<6 \text{ g}/100 \text{ g}$ carbohydrates, $<1 \text{ g}/100 \text{ g}$ fat) along with a small amount of oat granola (Øko Muesli, Kornkammeret, Lantmännen Cerealia A/S, Vejle, Denmark, $<62 \text{ g}/100 \text{ g}$ carbohydrates incl. $<13 \text{ g}/100 \text{ g}$ sugars, and $<6 \text{ g}/100 \text{ g}$ fat). Each participant had 2 ml/kg yogurt and one spoon of granola (5 gram) for all visits. On visits 2 and 4, a portion of $0.25 \times$ the daily dose of study liquid was mixed with the yogurt.

2.8. Samples and calculations

Blood samples were centrifuged at 3000rpm for 9 min at 4°C in EDTA-coated containers. Afterwards, the plasma was transferred to ice-cooled Eppendorf tubes, frozen on dry ice, and stored at -80°C for later analysis. Blood glucose and lactate were immediately analyzed in heparinized syringes on an ABL Flex system (Radiometer, Brønshøj, Denmark). The Department of Clinical Biochemistry at Rigshospitalet, Copenhagen, Denmark, analyzed myoglobin, creatine kinase (CK), and insulin on a COBAS 8000 (Roche, Basel, Switzerland).

Norepinephrine, epinephrine, and FFA were analyzed on a Wako NEFA-HR(2) assay kit (Wako Chemicals GmbH, Neuss, Germany) and a 2-CAT Plasma ELISA (Labor Diagnostika Nord GmbH, Nordhorn, Germany), respectively.

Breath samples were collected in a non-diffusible 15-liter Douglas bag (Hans Rudolph, Kansas, United States) and transferred to a vacutainer (BD, New Jersey, United States). Isotope enrichments of the used tracers, $C7\text{-ketones}$, and $^{13}CO_2$ were determined by gas chromatography-combustion-isotope ratio mass spectrometry (Finnigan MAT, Bremen, Germany) at Clinical Metabolomics Core Facility, Rigshospitalet, Copenhagen.

Palmitate and glucose R_a and R_d , palmitate oxidation rate, and total FAO were calculated as previously described [7]. An acetate correction factor was used to correct for the proportion of the labeled CO_2 that is produced via oxidation, that will not be excreted in blood or breath, but will be trapped in the tissues as described earlier [12].

2.9. Statistics

We calculated a sample size of 8 participants to detect a minimal relevant difference in the primary outcome measure,

Table 1
Demographics and baseline data on the 3 participating patients with phosphofructokinase deficiency.

Subjects:	TP01	TP02	TP03	Mean ± SD
Mutation	c.2003delC	c.450+1G>A	c.237+G>A	
Sex	F	F	M	
Age (years)	68	41	62	61 ± 13
BMI	17	26	25	23 ± 5
Maximal workload (watt)	28	47	58	44 ± 14
Submaximal workload (watt)	10	15	18	14 ± 4
VO _{2max} (ml × min ⁻¹ × kg ⁻¹)	13	15	18	16 ± 3
Physical activity (hours/week)	3	1	1	2 ± 1

M, male; F, female; BMI, body mass index; VO_{2max}, maximal oxidative capacity.

heart rate, during submaximal exercise of ≥ 5 bpm between active and placebo treatments. A power calculation was ($n = (\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\mu/\sigma})^2$) performed with regards to the primary endpoint heart rate during constant load cycling. This gave 80% power and two-sided 95% confidence intervals around assumed mean value for heart rate. A standard deviation (SD) of $5 \pm$ bpm for heart rate was assumed based on a previous study in patients with McArdle disease [13]. Because of recruitment challenges we ended up including only three participants. Results are presented as means \pm SE and statistical analyses were not performed due to the small sample size.

2.10. Approvals and registration

The study was approved by The Committee of Health Research Ethics of the Capital Region of Denmark (H-18,008,909) and the Danish Medicines Agency (2017–004,153–17). It was conducted within the ethical standards of the Declaration of Helsinki and monitored by the GCP unit at University of Copenhagen (Frederiksberg, Denmark). All participants gave written informed consent prior to inclusion.

2.11. Data availability

The data are not publicly available as they contain information that could compromise the privacy of the participants. Data are available on request from the corresponding author, DRP. The corresponding author had full access to the study data and had the final responsibility for the decision to submit for publication.

3. Results

3.1. Participants

Three patients with PFKD were included between August 2017 and August 2018. All three had a genetically confirmed diagnosis (Table 1), and reported symptoms of exercise intolerance with myalgia, contractures, and myoglobinuria. All participants completed a minimum of 10 days of treatment in both periods. Individual patient characteristics are listed in Table 1.

3.2. Standardized meal

Breakfast meals consisted on average of 118 ± 23 mL yogurt and one spoon of oat granola. Treatment liquid was added on visits 2 and 4. The breakfast took place on average $02:53 \pm 00:05$ hr:min before the exercise test.

3.3. Primary outcome

Triheptanoin did not improve the primary outcome measure heart rate during submaximal exercise compared to placebo (Fig. 1). In fact, two out of three participants had increased heart rate during exercise with triheptanoin treatment. Palmitate oxidation was increased during submaximal exercise in one patient, TP01, who also had a lower heart rate with triheptanoin treatment. Palmitate oxidation did not increase in the two other patients during triheptanoin treatment (Fig. 1).

3.4. Secondary outcome

Palmitate production (rate of appearance) and palmitate utilization (rate of disappearance) increased during exercise and increased to a greater extent with triheptanoin treatment compared to placebo both after submaximal exercise and after maximal exercise in all three patients (1.04 ± 0.96 vs. 2.20 ± 2.59 and 1.20 ± 1.06 vs. $2.06 \pm 2.38 \mu\text{mol} \times \text{kg}^{-1} \times \text{min}^{-1}$). Consistently, plasma palmitate and total free fatty acids concentrations increased during exercise and increased to a greater extent with triheptanoin during submaximal exercise in all three patients (Δ Palmitate concentration 85 ± 40 vs. 146 ± 92 and 255 ± 209 vs. $419 \pm 454 \mu\text{mol} \times \text{L}^{-1}$). Total FAO evaluated by indirect calorimetry also increased during exercise, however, the increase was similar during both treatments, both at rest and submaximal exercise (Fig. 2).

Glucose production (Ra) and utilization (Rd) increased immediately after exercise start and peaked after five minutes of exercise, followed by a decline during the remaining submaximal exercise with both triheptanoin treatment and placebo in all three patients. Accordingly, total carbohydrate

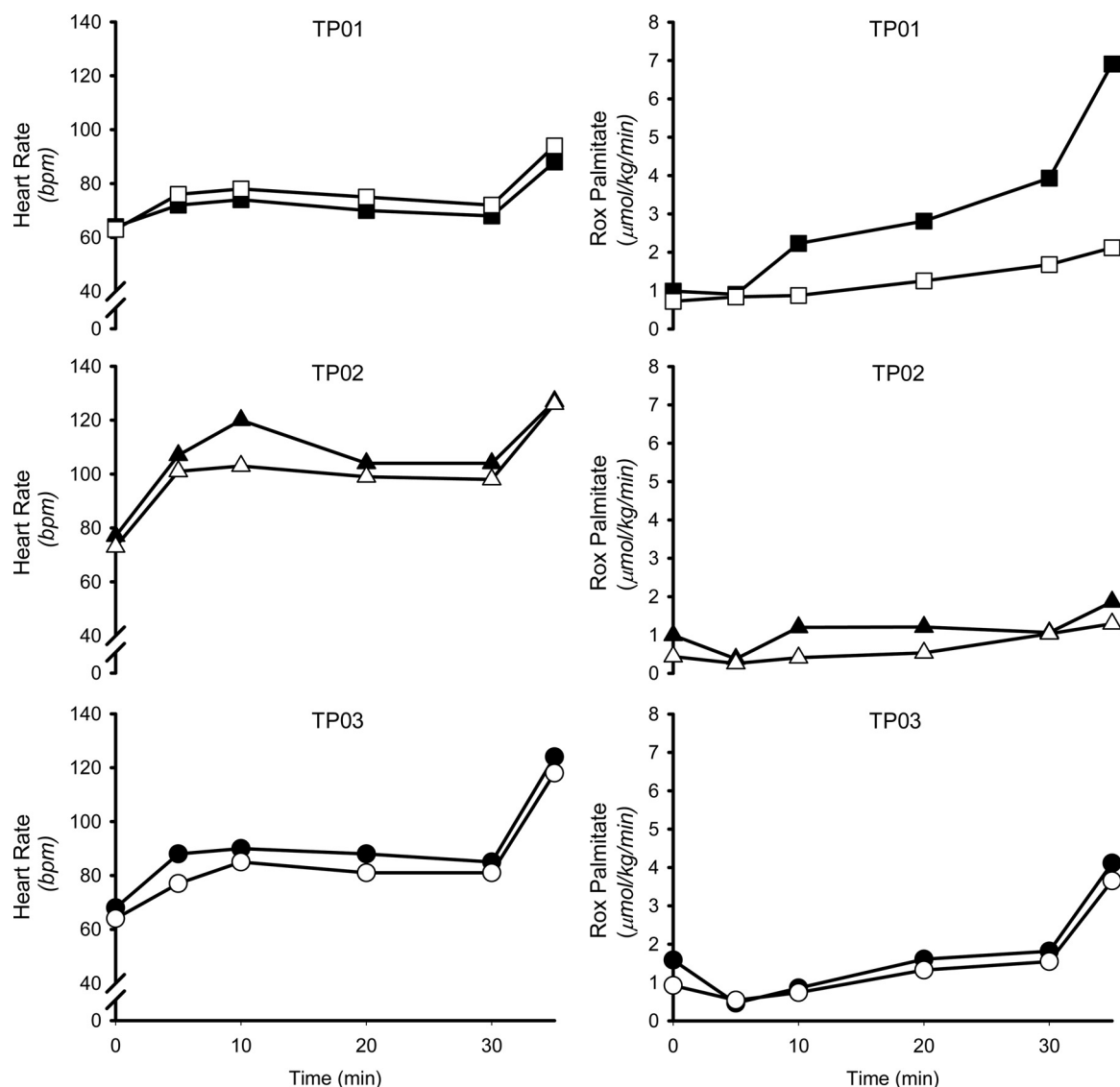


Fig. 1. Primary outcome measures: Heart rate and palmitate rate of oxidation (Rox) during submaximal (30min) to maximal exercise in 3 PFKD patients after treatment with placebo □ and triheptanoin ■ for 14 days in a double-blind crossover study.

oxidation (CHO) increased slightly during submaximal exercise with both treatments in all three patients (Fig. 3).

Exercise duration was longer with placebo vs triheptanoin ($35:03 \pm 03:19$ vs. $35:40 \pm 02:09$). However, mean perceived exertion during submaximal exercise was comparable between treatments (Fig. 4).

There was no effect of triheptanoin on maximal workload nor maximal oxidative capacity in any of the patients (40 ± 15 vs. 42 ± 14 W placebo and 14 ± 4 vs. 14 ± 2 $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$) (Fig. 4). Both workload and maximal oxygen uptake were severely reduced compared to healthy individuals at same age (Women:men, 92 ± 19 vs. 162 ± 38 W and 20.5 vs. 29.9 $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$, 90 percentile) [14].

Both the SF-36 form and the Bouchards energy expenditure questionnaire showed no improvements with

triheptanoin treatment (-4 ± 3.5 , $n=2$; and -6.5 ± 13.7 METs day^{-1} , $n=3$)

3.5. Metabolites and hormones

At rest, blood glucose levels were higher on placebo. At maximal exercise, blood glucose was at same level on both treatments (Fig. 3). Blood lactate levels decreased from start to end of exercise on both treatments, most pronounced with placebo (-0.08 ± 0.27 vs. -0.02 ± 0.23 $\text{mmol} \times \text{L}^{-1}$). FFA concentrations during exercise increased to a greater extent on triheptanoin vs. placebo (Table 2). Adrenaline levels increased to a higher level on triheptanoin (112 ± 32 vs. 206 ± 283 $\text{pg} \times \text{ml}^{-1}$). At the end of exercise, ammonium levels were higher on placebo vs. triheptanoin (125 ± 33 vs. 93 ± 74 $\mu\text{mol} \times \text{L}^{-1}$, $n=2$).

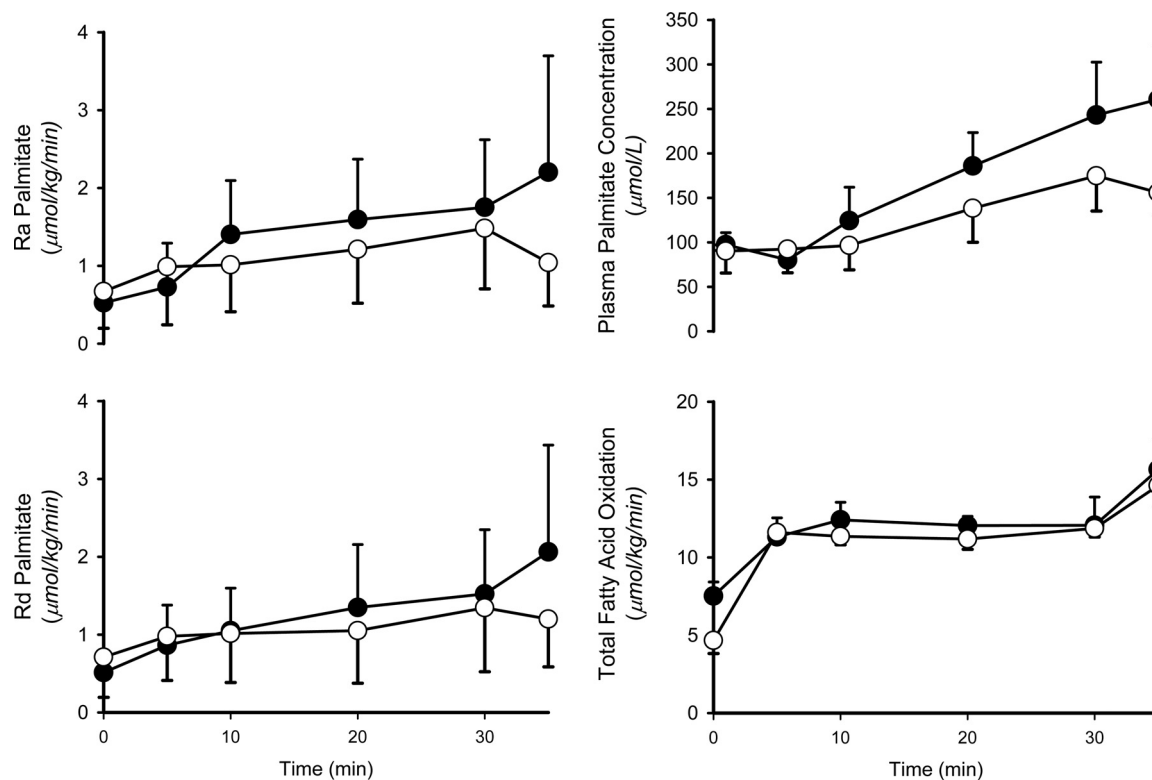


Fig. 2. Palmitate rate of appearance (Ra) and rate of disappearance (Rd), plasma palmitate concentration and total fat oxidation during submaximal (30 min) to maximal exercise in 3 PFKD patients after treatment with placebo ○ and triheptanoin ● for 14 days in a double-blinded crossover study. Values are mean with standard error of the mean as bars.

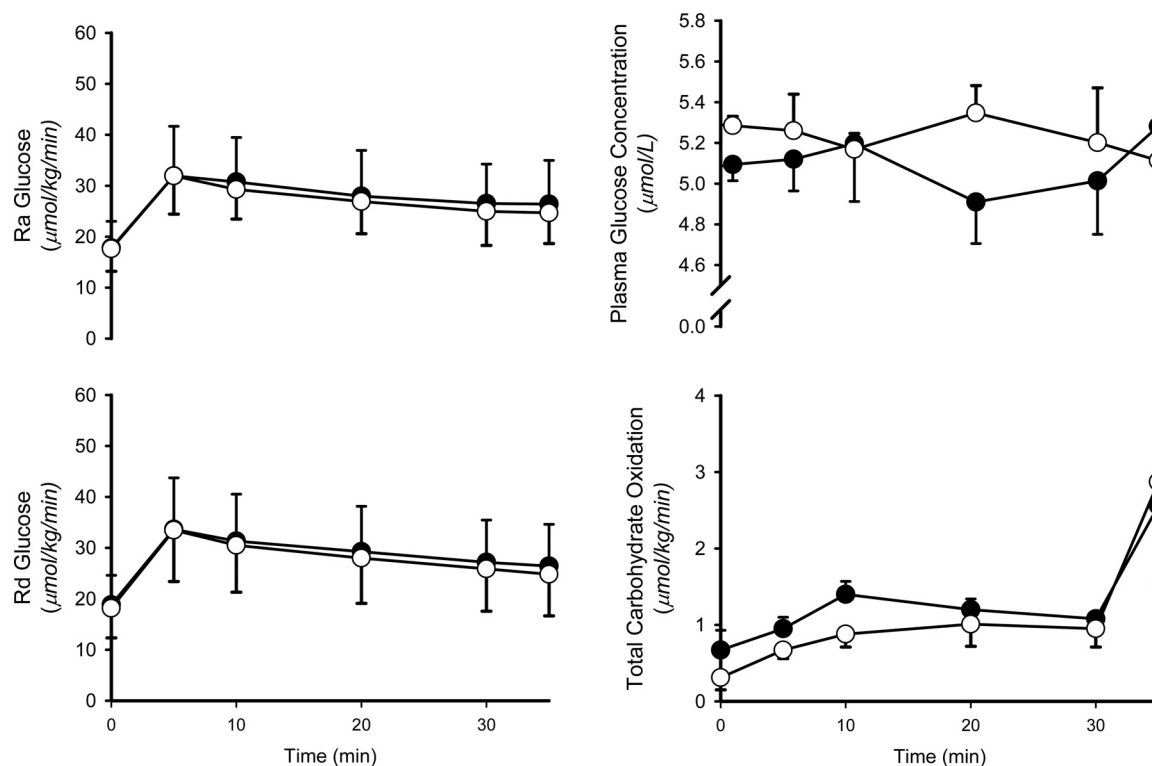


Fig. 3. Glucose rate of appearance (Ra) and rate of disappearance (Rd), plasma glucose concentration and total carbohydrate oxidation during submaximal (30 min) to maximal exercise in 3 PFKD patients after treatment with placebo ○ and triheptanoin ● for 14 days in a double-blinded crossover study. Values are mean with standard error of the mean as bars.

Table 2

Plasma metabolites at rest and submaximal exercise in three patients with phosphofructokinase deficiency. Submaximal values are mean of blood samples drawn at 5, 10, 20, and 30 min after the onset of exercise with standard error of the mean.

		Triheptanoin			Placebo		
		TP01	TP02	TP03	TP01	TP02	TP03
FFA ($\mu\text{mol L}^{-1}$)	Rest	488	447	432	393	302	593
	Submaximal	907 \pm 194	423 \pm 19	563 \pm 107	704 \pm 91	332 \pm 29	570 \pm 65
Lactate (mmol L^{-1})	Rest	0.6	0.7	0.4	0.5	0.9	0.5
	Submaximal	0.6 \pm 0.01	0.5 \pm 0.03	0.4 \pm 0.01	0.5 \pm 0.01	0.7 \pm 0.06	0.5 \pm 0.01
C7 ($\mu\text{mol L}^{-1}$)	Rest	5.7	14.6	81.2	15.9	2.9	4.0
	Submaximal	4.2 \pm 0.7	32.6 \pm 3.7	22.7 \pm 10.2	9.4 \pm 4.4	3.2 \pm 0.2	4.9 \pm 0.8
Pyruvate ($\mu\text{mol L}^{-1}$)	Rest	54	112	59	65	114	59
	Submaximal	52 \pm 5	87 \pm 9	63 \pm 5	54 \pm 2	59 \pm 8	56 \pm 1
HOB ($\mu\text{mol L}^{-1}$)	Rest	97	97	168	52	73	292
	Submaximal	105 \pm 8	100 \pm 5	189 \pm 17	83 \pm 11	64 \pm 11	267 \pm 11

FFA. Free fatty acids; C7, heptanoate; HOB, β -hydroxybutyrate;.

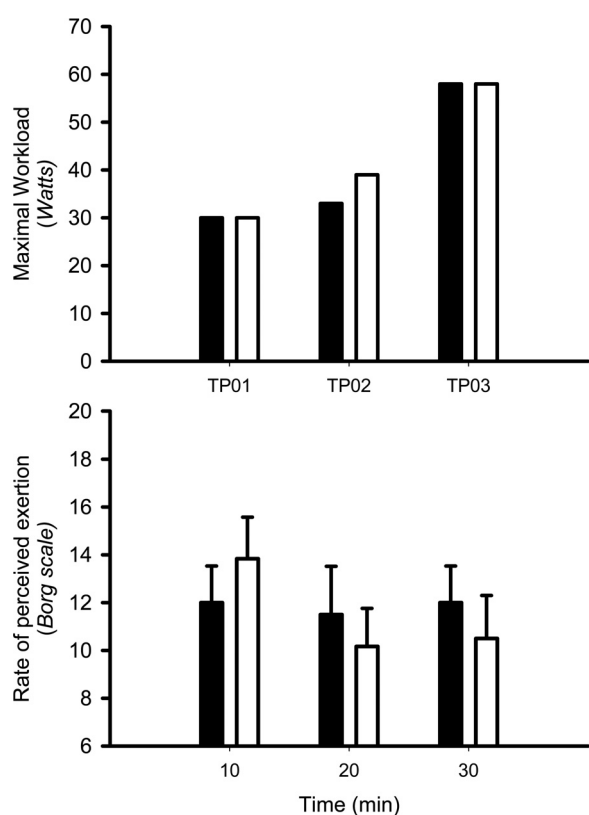


Fig. 4. Perceived exertion and maximal workload during exercise in 3 PFKD patients after treatment with placebo \square and triheptanoin \blacksquare for 14 days in a double-blinded crossover study. Values are mean of a two minutes interval with standard error of the mean as bars for rates of perceived exertion, and values are absolute of end-exercise workload for maximal workload.

Safety parameters: Before exercise, CK and myoglobin concentrations were 383 ± 253 for placebo vs. $470 \pm 337 \text{ U} \times \text{L}^{-1}$ for triheptanoin, and 80 ± 54 for placebo vs. $84 \pm 31 \mu\text{g} \times \text{L}^{-1}$ for triheptanoin.

At onset of exercise, pyruvate levels decreased after the first 20 min of exercise and then stabilized and did not change until end of exercise in both treatments (Table 2). β -hydroxybutyrate (HOB) levels also decreased during the

first 10 min but increased afterwards to a higher level than rest values on both treatments (Table 2). In two patients C7 levels were 4- and 5-fold higher during rest and submaximal exercise on triheptanoin compared to placebo (Table 2).

3.6. Side effects and compliance

On placebo, participants' body weight changed by a mean of $-0.9 \pm -0.3 \text{ kg}$. On triheptanoin, no change in body weight was found.

During the trial period, no serious adverse events were reported. One participant had a bacterial dental infection. The infection occurred during triheptanoin treatment, was treated with antibiotics, and was not considered as treatment-related by the investigators. Rhabdomyolysis or myoglobinuria did not occur in relation to exercise tests or at any other time during study period. One of three participants reported periods of nausea and diarrhea on the placebo treatment. When side effects occurred, participants were guided to reduce the dose, which solved the problem. No side effects were reported on the triheptanoin treatment. Thus, there was no reduction of triheptanoin treatment at any time.

After each treatment period, the collected remaining liquid indicated a drug compliance of 82–100% on placebo and 98–100% on triheptanoin. Compliance to the treatment was also reaffirmed by the increase in C7 compared to placebo (Table 2).

4. Discussion

The main findings of the study are: 1) A 14-day triheptanoin treatment (30% of total calories intake) had no effect on the primary outcome measures: lowering of heart rate and improvement of fat oxidation during constant workload cycle exercise compared to placebo. 2) Triheptanoin treatment increased plasma palmitate production and thus plasma palmitate and plasma FFA concentrations in all three patients with PFKD during exercise. 3) Glucose production, utilization and oxidation were unchanged after triheptanoin treatment vs. placebo. 4) Triheptanoin did not improve

perceived exertion as well as VO_{2max} and W_{max} during constant load cycling. 5) Finally, patients did not feel better during triheptanoin treatment as reflected in the reported questionnaires of health (SF-36) and energy expenditure (Bouchards).

To our knowledge, this is the first study to investigate fat and glucose metabolism during rest and exercise in patients with PFKD with the use of stable isotope techniques. From rest to maximal exercise, all participants had an increase of substrates for fat metabolism and a parallel increase in total FAO, comparable to findings in patients with McArdle disease (Fig. 2) [7]. Yet, there are differences in the levels achieved. Both production and oxidation of palmitate is up to 2-fold lower in PFKD, and palmitate oxidation and total FAO are equally reduced compared to McArdle disease [7]. These findings are in accordance with the more severe clinical presentation of PFKD.

ATP is the main source of energy at rest and during exercise. It is a product of oxidation of blood glucose and FFA. Exercise intensity and duration determine the relative amounts of FFA, muscle glycogen, and blood glucose that are oxidized next to alternative sources such as the minor contributors like ketone bodies, lactate, and amino acids. At low intensity of longer duration (<65% of VO_{2max}), FFA is the primary substrate used for ATP-generation, while high-intensity exercise (>75% of VO_{2max}) mainly use muscle glycogen and to a lesser extent blood derived glucose [15]. In fact, in healthy subjects fat oxidation will decrease, when intensity increases [16]. Even though triheptanoin did not improve exercise tolerance, it seemed to influence fat metabolism by increasing the concentration of FFA and palmitate in all patients and the rate of palmitate oxidation in one patient (Fig. 2 and Table 2). However, this increased fat metabolism is not enough to improve exercise intolerance and a considerable energy disturbance still exists.

Given the rarity of PFKD, not many studies have been conducted on possible therapies to this date. Studies on the more prevalent McArdle disease have been used as a beacon for treatment recommendation as the two GSDs share common manifestations. However, one distinct feature between these metabolic deficiencies is the actual ability to metabolize glucose in McArdle disease but not in PFKD. During exercise, McArdle patients are therefore likely to use bloodborne glucose from hepatic glycogenolysis to fuel the TCA cycle with pyruvate-derived oxaloacetate. Two studies have shown low levels of TCA intermediates (TCAI) during exercise in McArdle disease compared to healthy controls [17,18]. Thus, in the case of PFKD, we anticipated even lower levels of TCAI. Therefore, we expected a greater benefit of triheptanoin treatment in PFKD patients.

Recently, our laboratory completed a controlled trial with triheptanoin treatment in patients with McArdle disease showing no effects on exercise performance measurements [19]. In this study, exercise started within 90 min after liquid ingestion. Although, Madsen et al. observed an increase in TCAI in the form of malate at rest and during exercise compared to placebo, it did not translate into improved

exercise performance or changes in respiratory quotient. In addition, no further increase in malate concentration was observed during exercise as seen in healthy individuals [17]. Similarly, the hypothesized alleviating effect of anaplerosis on the TCAI-limited oxidative phosphorylation in PFKD was not observed in the present study, which mimics the results found in McArdle disease [19]. This poses the question of whether triheptanoin derived C5-metabolites are available for muscle in proper concentrations or if they are used by other ketone-consuming tissues, such as the kidney or brain, or if the limitation of exercise performance is not due to lack of TCAIs.

This could also be explained by compliance problems or too low dose of triheptanoin. However, C7 levels in the PFK patients were increased by triheptanoin supplementation at rest and during exercise compared to placebo in two patients, indicating appropriate compliance, while the third patient had greatest increase in palmitate oxidation (Fig. 1 and Table 2). Furthermore, dose levels of triheptanoin were higher or similar compared to previous studies [20–22]. A study by Roe et al. of 3 patients with VLCAD, showed maximum C7 and odd-carbon ketone bodies concentrations were reached at 90–150 min after meal ingestion [23]. In our study patients ingested the triheptanoin containing meal 150–180 min before exercise, using a more acute treatment protocol might have shown even better results in the FAO parameters, although exercise performance was not improved in patients with McArdle disease by triheptanoin supplementation between 30 and 60 min before exercise [19].

The diet in this study $1\text{g} \times \text{kg}^{-1} \times \text{day}^{-1}$ was comprehensive as the liquid constituted a third of the total caloric intake, could not be heated, and participants had to avoid a range of everyday foods. Before exercise start, each participant had the liquid with their standardized breakfast. However, the effect of food ingestion on carbohydrate and fat metabolism must be considered when using stable isotopes. An increase in blood glucose will block endogenous glucose production [24]. Still, we anticipated a similar baseline metabolic state during the tests as the breakfast was thoroughly copied between tests. Ingestion of the liquid alone before exercise start would not be possible due to side effects. Although, none of the participants reported side effects, they have been frequently reported elsewhere [19,22]. In general, implementing a triheptanoin diet challenge compliance of the patients and could be a problem taking into account our results with no observable clinical effect, price of the treatment and the caloric load of the diet. Increasing the amount of daily intake of triheptanoin liquid most likely is not possible for the patients. In this study recruitment was limited to only three patients, however, results were consistent in all three patients with triheptanoin not improving exercise tolerance, and it is difficult to envision that including more patients would change that picture.

Currently, there are no additional treatment trials registered at clinicaltrials.gov for patients with PFKD. Future trials should consider broad international collaboration with neuromuscular centers to facilitate the recruitment process and

design of study. Moreover, trials should consider partnerships with patient organization. In the future, a solution for PFKD is gene therapy, and now, gene therapies for other GSDs are studied in early phase clinical trials [25]. There are no studies specifically focused on PFKD, but results in a murine model for McArdle are promising [26]. However, at this moment in time, it is known that both fasting and triglyceride-heparin infusion improves exercise performance in patients with PFKD [6,25]. A recent study also reports subjective alleviation of muscle pain symptoms and better exercise tolerance in a patient with PFKD on a ketogenic diet – a diet characterized by achieving ketosis with high levels of bloodborne ketones [27]. These findings are supported by a pilot study on ketogenic diet in patients with McArdle disease showing improved FAO and exercise tolerance [28]. In line with this, our patients recognized from their daily lives that fasting improves exercise performance, could suggest a place for ketone body therapy in PFKD. Thus, a ketogenic diet could be a possible treatment option for PFKD today.

5. Conclusion

The present study demonstrates that fat metabolism does not reach same levels in patients with PFKD during exercise as seen in previous studies on patients with McArdle disease. Further, our study indicates that a 14-day triheptanoin treatment does not improve exercise performance, measured as a reduced heart rate or fat metabolism. The treatment increased plasma C7 levels in 2 of 3 patients, but concentrations were still low in terms of absolute quantities and resulted in a relative limited increase in plasma C7 upon the start of exercise. Thus, triheptanoin used as a day round ergogenic acid does not improve exercise performance in patients with PFKD.

Details of the contributions of individual authors

DRP conceptualized and designed the study, collected, analysed, and interpreted the data, and drafted the manuscript and figures. RQ, PL, AL, JV and KLM, conceptualized and designed the study, played a major role in acquisition of data. JHS and NL, analysed the data and played a major role in acquisition of data. GH conceptualized and designed the study, analysed, and interpreted the data. MØ conceptualized and designed the study, collected, analysed, and interpreted the data, guarantor for the article. All authors contributed to the final drafting and review of the manuscript.

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Declaration of Competing Interest

DRP, MØ, NL, JHS, AL, GV, JV, PL and KLM declare no potential conflicts of interest. RQ has been part of an advisory board at Ultragenyx Pharmaceuticals Inc not related to the disease, study, or interventions.

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Further reading

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