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Medium-chain triglycerides impair lipid metabolism and induce hepatic steatosis in very long-chain acyl-CoA dehydrogenase (VLCAD)-deficient mice

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ABSTRACT

A medium-chain-triglyceride (MCT)-based diet is mainstay of treatment in very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD), a long-chain fatty acid β-oxidation defect. Beneficial effects have been reported with an MCT-bolus prior to exercise. Little is known about the impact of a long-term MCT diet on hepatic lipid metabolism. Here we investigate the effects of MCT-supplementation on liver and blood lipids in the murine model of VLCADD.

Wild-type (WT) and VLCAD-knock-out (KO) mice were fed (1) a long-chain triglyceride (LCT)-diet over 5 weeks, (2) an MCT diet over 5 weeks and (3) an LCT diet plus MCT-bolus. Blood and liver lipid content were determined. Expression of genes regulating lipogenesis was analyzed by RT-PCR.

Under the LCT diet, VLCAD-KO mice accumulated significantly higher blood cholesterol concentrations compared to WT mice. The MCT-diet induced severe hepatic steatosis, significantly higher serum free fatty acids and impaired hepatic lipid mobilization in VLCAD-KO mice. Expression at mRNA level of hepatic lipogenic genes was up-regulated.

The long-term MCT diet stimulates lipogenesis and impairs hepatic lipid metabolism in VLCAD-KO mice. These results suggest a critical reconsideration of a long-term MCT-modified diet in human VLCADD. In contrast, MCT in situations of increased energy demand appears to be a safer treatment alternative.

Introduction

Mitochondrial β-oxidation of fatty acids is an important process for provision of energy necessary for a wide variety of metabolic reactions. Therefore, its dysfunctions, e.g. due to inherited enzyme defects may have severe consequences on human health with involvement of different organ systems. Deficiency of the very long-chain acyl-CoA dehydrogenase (VLCAD) is the most common β-oxidation disorder of long-chain fatty acids with an incidence of 1:55.000 to 1:100.000 births [1]. The clinical phenotype of VLCAD-deficiency (VLCADD) is very heterogeneous and presents with different severity and age of onset [1]. Before newborn screening for fatty acid oxidation disorders was implemented, the most severe phenotype became manifest in the first weeks and months of life with cardiomyopathy and hepatopathy. The infantile hepatic phenotype presented with hypoketotic hypoglycemia and lethargy and was triggered by preceding infections. The milder later-onset myopathic phenotype became usually manifest in adolescence or young adulthood with muscle weakness, rhabdomyolysis and myoglobinuria [2,3]. Overall, fasting and other catabolic stress as results of illnesses or prolonged exercise may induce or aggravate clinical symptoms and under these conditions severe metabolic derangement may occur.

VLCAD-deficient patients are unable to oxidize fatty acids originating from the diet or endogenous lipolysis, which subsequently accumulate as long-chain acyl-CoAs and acyl-carnitines [4]. So far, treatment recommendations for VLCADD include the avoidance of fasting and a fat-modified and long-chain triglyceride (LCT)-restricted diet [5]. Fat modification includes the replacement of LCT by medium-chain triglycerides (MCT). In fact, it is generally accepted that, medium-chain fatty acids can cross the mitochondrial membrane as carboxylates without previous esterification into acyl-carnitines by carnitine palmitoyl-CoA transferases (CPT I) and can be fully metabolized [5,6].

A rather novel dietary approach with MCT has been reported to have great beneficial effects during periods of increased energy

Abbreviations: VLCADD, very-long-chain acyl-CoA dehydrogenase deficiency; VLCAD KO, very-long-chain acyl-CoA dehydrogenase knock-out; WT, wild type; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; ACC-1α, acyl-CoA carboxylase; FASN, fatty acid synthase; 1 SREBP-1c, sterol regulatory element binding transcription factor; SCD1, stearoyl-Coenzyme A desaturase; TGA, triglyceride; FFA, free fatty acids.

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demand. In this case, MCT is administered as bolus immediately prior to exercise in patients with VLCADD, carnitine palmitoyl-CoA transferase II (CPT II)-deficiency and long-chain hydroxy acyl-CoA-dehydrogenase deficiency (LCHADD) [7–9]. Despite the positive reports of MCT treatment to maintain metabolic control in a number of patients with different long-chain fatty acid oxidation defects, there have also been scattered reports that point to adverse effects of MCT-supplementation on liver function of patients with intractable epilepsy [10]. Therefore, the actual impact on lipid homeostasis and lipid clearance, especially in VLCADD, remains to be tested.

To study the effects of dietary interventions, the VLCAD knockout (KO) mouse represents an excellent animal model as it displays a very similar clinical phenotype to human VLCADD [4]. For instance, in the absence of stress VLCAD-KO mice appear asymptomatic but triggers such as fasting, cold exposure and physical exercise give rise to accumulation of long-chain acyl-carnitines (e.g. C16:0 and C18:1) and result in hypoglycaemia, hepatopathy and skeletal myopathy [11,12].

To address the question how MCT affects lipid homeostasis and lipid clearance, we studied wild-type (WT) and VLCAD-KO mice fed with a long-term MCT diet and an MCT-bolus treatment, in an effort to compare dietary effects with a normal LCT diet. To gain insight into fat metabolism under different stress conditions, mice were submitted to different workloads. We analyzed liver and blood lipid concentrations at rest, after physical exercise, and after 24 h of regeneration following exercise. Furthermore, we analyzed the effects of the MCT-based diet on the expression of genes regulating *de novo* biosynthesis and elongation of fatty acids. Our results clearly demonstrate that the long-term MCT diet in VLCAD-KO mice has a profound impact on lipid mobilization and clearance.

Materials and methods

Reagents

All chemicals used were purchased in p.a. quality from J.T. Backer (Griesheim, Germany), Merck (Darmstadt, Germany), Riedel de Haën (Seelze, Germany), Roche (Penzberg, Germany), and Sigma–Aldrich (Deisenhofen, Germany).

Animals

VLCAD-KO mice used in these studies were kindly provided by A.W. Strauss (currently Cincinnati Children's Hospital, Ohio, USA) and were generated as described in detail previously [13]. Experiments were performed on fourth- to fifth-generation intercrosses of C57BL6+129sv VLCAD genotypes. Littermates served as controls and genotyping of mice was performed as described previously [13].

Groups consisting of five mice aged 10–12 week-old were analyzed (1) under well-fed, resting conditions, (2) after short-term exercise of 1 h and (3) after 24 h regeneration following exercise. Blood samples were collected by heart puncture and serum was obtained by centrifugation at 16,000*g* for 10 min and stored at −80 °C for further analysis. The mice were either sacrificed immediately prior, after exercise or 24 h after the exercise protocol was terminated. Liver was rapidly removed and immediately frozen in liquid nitrogen.

All animal studies were performed with the approval of the Heinrich-Heine-University Institutional Animal Care and Use Committee. The care of the animals was in accordance with the Heinrich-Heine-University Medical Center and Institutional Animal Care and Use Committee guidelines.

Diet composition and supplementation

After weaning, at ~5–7 weeks of age, mice were divided in three groups and fed with different diets for 5 weeks. The first group received a purified mouse diet containing 5.1% crude fat in form of LCT, corresponding to 13% of metabolizable energy calculated with Atwater factors (ssniff® EF R/M Control, ssniff Spezialdiäten GmbH, Soest, Germany). The second group was fed with a diet (ssniff® EF R/M control, ssniff GmbH, Soest, Germany) corresponding, as well, to ca. 13% of total metabolizable energy calculated with Atwater factors, in which 4.4% from a total of 5% fat, were MCT- (Ceres®MCT-oil, basis GmbH, Oberpfaffenhofen, Germany) while the remaining 0.6% derived from the essential soy bean oil. In both diets the carbohydrate and protein contents were unmodified and corresponded to 65% and 22% of metabolizable energy, respectively. A third group received long term the same LCT diet as group 1 but was supplemented in addition half an hour prior to exercise a single oral MCT-oil bolus of 2 g/kg body weight.

Exercise protocol

As mice are nocturnal animals treadmill running was performed during the dark cycle. Ten to twelve weeks old WT and VLCAD-KO mice were exercised 60 min on a Columbus Instruments Simplex II metabolic rodent treadmill consisting of four individual lanes without inclination and a shock plate incentive (3 Hz, 200 ms, 160 V, 1.5 mA). The training protocol was performed as already described by Ter Veld et al. [14] with minor modifications. Briefly, mice were placed in an exercise chamber, and after an adaptation period of 15 min, initial belt speed was set to 4 m/min and increased every 5 min by 2 m/min to a maximum of 16 m/min. Mice ran until they displayed signs of exhaustion (>2 s spent on the shocker plate without attempting to re-engage the treadmill).

Lipid and lipoprotein analysis

Serum was collected 90 min from the last meal. Free fatty acid and lipoprotein concentrations were measured as duplicates in serum samples by using enzymatic kits (free fatty acid quantification kit, Biocat, Heidelberg, Germany; EnzyChrom HDL and VLDL/LDL Assay kit, BioTrend, Cologne, Germany) on an Infinite M200 Tecan (Crailsheim, Germany) plate reader. Serum TGAs and liver TGAs were measured as duplicates by using enzymatic kits (EnzyChrom Triglyceride Assay kit BioTrend Cologne, Germany and Triglyceride Quantification kit, Biocat, Heidelberg, Germany, respectively). All assays were performed following the manufacturer's instructions.

Intrahepatic lipid content

The intrahepatic lipid content was measured gravimetrically. Lipids were extracted according to a method by Folch et al. [15] modified as follows. Lyophilized liver (20–40 mg) was homogenized in 0.5 mL distilled water and extracted in 4 mL chloroform/methanol ($CHCl_3$/MeOH) 2:1 (v/v) by gentle agitation at 4 °C for 3 h. 0.8 mL of 0.9% NaCl was added to the mixture, shaken vigorously and centrifuged at 2500*g* for 10 min. The organic phase containing the extracted lipids was removed and evaporated under nitrogen flow at 40 °C.

RT-PCR analysis

Total liver RNA was isolated with the RNeasy mini kit (Qiagen, Hilden Germany). Forward and reverse primers for β-actin (BC138614), fatty acid synthase (FASN; NM_007988.3), acyl-CoA