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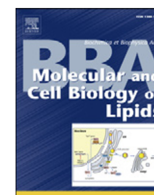

Hepatic fatty acid uptake is regulated by the sphingolipid acyl chain length

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ABSTRACT

Ceramide synthase 2 (CerS2) null mice cannot synthesize very-long acyl chain (C22–C24) ceramides resulting in significant alterations in the acyl chain composition of sphingolipids. We now demonstrate that hepatic triacylglycerol (TG) levels are reduced in the liver but not in the adipose tissue or skeletal muscle of the CerS2 null mouse, both before and after feeding with a high fat diet (HFD), where no weight gain was observed and large hepatic nodules appeared. Uptake of both BODIPY-palmitate and [^{3}H]-palmitate was also abrogated in the hepatocytes and liver. The role of a number of key proteins involved in fatty acid uptake was examined, including FATP5, CD36/FAT, FABPpm and cytoplasmic FABP1. Levels of FATP5 and FABP1 were decreased in the CerS2 null mouse liver, whereas CD36/FAT levels were significantly elevated and CD36/FAT was also mislocalized upon insulin treatment. Moreover, treatment of hepatocytes with C22–C24-ceramides down-regulated CD36/FAT levels. Infection of CerS2 null mice with recombinant adeno-associated virus (rAAV)-CerS2 restored normal TG levels and corrected the mislocalization of CD36/FAT, but had no effect on the intracellular localization or levels of FATP5 or FABP1. Together, these results demonstrate that hepatic fatty acid uptake via CD36/FAT can be regulated by altering the acyl chain composition of sphingolipids.

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1. Introduction

Triacylglycerols (TG) are transported in very low density lipoprotein (VLDL) particles or chylomicrons and degraded to free fatty acids (FFAs) by the action of hepatic lipase (HL). Although FFAs were thought to enter cells by diffusion, long chain fatty acid (LCFA) uptake is now believed to be, at least partially, protein-mediated, dependent on fatty acid transport proteins (FATPs), plasma membrane fatty acid binding protein (FABPpm) and fatty acid translocase (CD36/FAT) [1,2]. Six FATP family members have been identified, with each displaying tissue-specific expression [1]. An FATP5 null mouse displays reduced hepatic TG and FFA levels [3]. Upon insulin stimulation, CD36/FAT localizes from an interior vesicle population to the plasma membrane [4–7], and in the absence of caveolin-1, CD36/FAT is mis-targeted out of detergent-resistant membranes (DRMs), resulting in reduced FFA uptake [8]. A caveolin-1 null mouse is resistant to diet-induced obesity [9], and inhibition of caveolae formation results in decreased oleic acid uptake [10].

Sphingolipids (SLs) are major components of the plasma membrane where they are involved in regulating a number of processes. The backbone of all SLs is ceramide. In mammals, ceramide is synthesized by six ceramide synthases (CerS) [11], with each using acyl CoAs of different chain lengths for *N*-acylation of the sphingoid long chain base. CerS2, which generates very-long acyl chain (VLC) ceramides (C22–24-ceramides), is the best characterized CerS, largely due to the generation and characterization of a CerS2 null mouse [12,13], which displays a wide range of pathologies, many of which are associated with the liver, where CerS2 is expressed at high levels. CerS2 null mice contain virtually no VLC-ceramides but contain elevated levels of C16-ceramide and sphinganine, which appear to be the cause of hepatopathy, hepatic insulin resistance, hepatic oxidative stress and mitochondrial dysfunction [12–15], probably due to altered membrane properties [16]. However, the pathologies are not caused by a general defect in liver function, but rather by the dysfunction of specific biochemical pathways. For instance, CerS2 null mice are insensitive to LPS/galactosamine-mediated

Abbreviations: CD36/FAT, fatty acid translocase; CerS, ceramide synthase; DRMs, detergent resistant membranes; FABP, fatty acid binding protein; FABPpm, plasma membrane fatty acid binding protein; FATP, fatty acid transport protein; FFA, free fatty acid; HFD, high fat diet; LCFA, long chain fatty acid; NAFLD, nonalcoholic fatty liver disease; SLs, sphingolipids; SSO, sulfo-N-succinimidyl oleate; TG, triacylglycerol; VLC, very-long chain

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hepatic failure due to defective TNFR1 internalization [17] and to drug-induced liver injury due to dysfunctional gap junctions [18]. Thus, it is not possible to predict the outcome of manipulation of the liver due to the variety of pathways that are, or are not affected, by altering the SL acyl chain composition.

We now demonstrate that LCFA uptake is disrupted in the CerS2 null mouse liver, resulting in reduced hepatic TG accumulation even after a high fat diet (HFD). These data might implicate SL synthesis as a putative regulatory mechanism in nonalcoholic fatty liver disease (NAFLD).

2. Materials and methods

2.1. Materials

Optiprep, tyloxapol and sulfo-N-succinimidyl oleate (SSO) were from Sigma Aldrich (St Louis, MO). C16–C24 ceramides were from Avanti Polar Lipids (Alabaster, AL). The antibodies used in this study were anti-caveolin-1, anti-clathrin (Cell Signaling Technology, Beverly, MA), anti-flotillin-1, anti-IRβ (BD Biosciences, San Diego, CA), anti-α-tubulin, anti-β-actin, anti-HA, anti-Flag, anti-CerS2 (Sigma Aldrich, St Louise, MO), anti-FATP5, anti-1163-phosphorylated IRβ (Santa Cruz Biotechnology, Santa Cruz, CA), anti-CD36/FAT (eBioscience, San Diego, CA), anti-GAPDH (Millipore, Temecula, CA), anti-FABPpm (Abcam, Cambridge, MA) and anti-Cy2, anti-Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA). The anti-FABP1 antibody was obtained as described [19]. [9,10-^{3}H(N)]-triolein and [^{14}C]-palmitic acid were from the American Radiolabeled Chemicals (St Louis, MO) and [9,10-^{3}H (N)]-palmitic acid was from Amersham International (Amersham, UK). BODIPY-palmitate was from Invitrogen (Carlsbad, CA).

2.2. Animals

CerS2 null mice were generated as described [12,13]. All mice were treated according to the Animal Care Guidelines of the Weizmann Institute of Science Animal Care Committee and the National Institutes of Health's Guidelines for Animal Care.

2.3. Lipid extraction and thin layer chromatography

Lipids were extracted using chloroform/methanol, (2:1, vol:vol) and separated by thin layer chromatography (TLC) using heptane/isopropyl ether/acetic acid as the developing solvent (60:40:3, vol:vol:vol). TLC plates were sprayed with copper sulfate (15.6% copper sulfate, 9.4% phosphoric acid) and incubated at 100 °C for 8 min.

2.4. TG and FFA quantification

TGs and FFAs were measured using colorimetric assays (Triglyceride Quantification Kit, Biovision, Palo Alto, CA, and Free Fatty Acid Quantification kit, Biovision, Palo Alto, CA).

2.5. High fat diet

HFD (D12492) (60% fat, 20% carbohydrate, 20% protein, 5.24 kcal/g) and chow diets (D12450K) (10% fat, 70% carbohydrate, 20% protein,

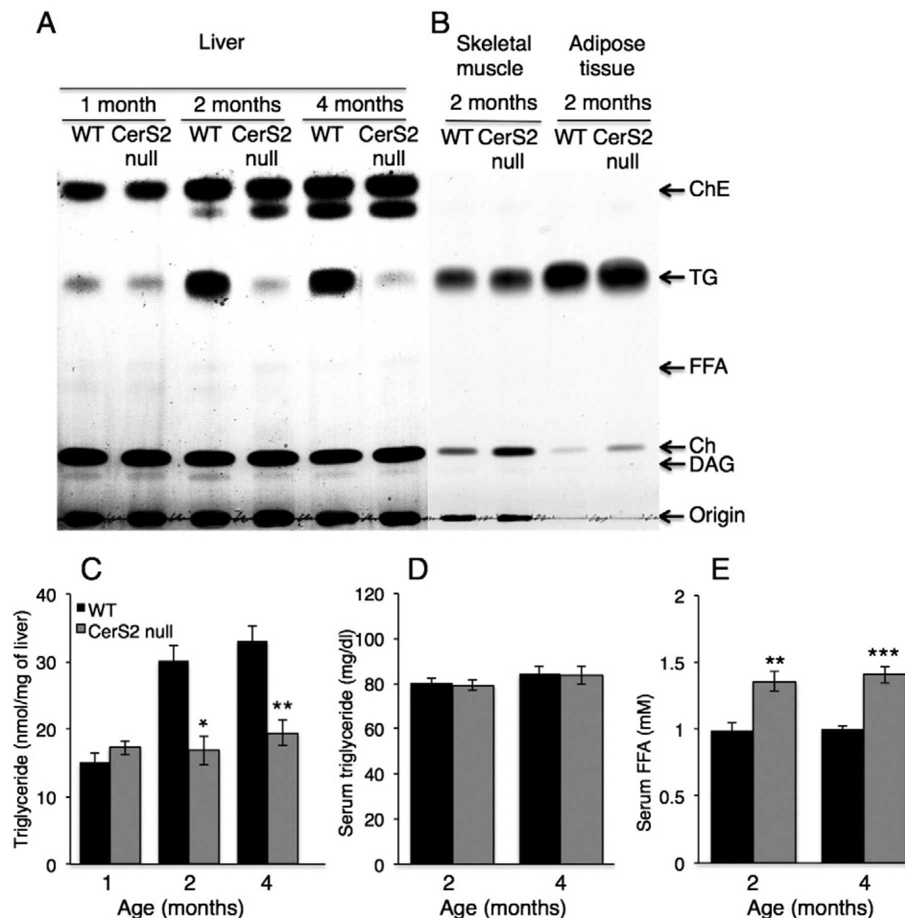

Fig. 1. TG and FFA levels in 1–4 month-old CerS2 null mouse liver. TG levels were measured in the liver by two independent methods, (A) TLC (representative result of 3 independent experiments) and (C) a colorimetric assay (n = 3). (B) Lipid levels in skeletal muscle and adipose tissue. (D) TG and (E) FFA levels in serum (n = 5–6). ChE, cholesterol ester; Ch, cholesterol; DAG, diacylglycerol. Data are means ± S.E.M. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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