Published in final edited form as:

Diabetes Obes Metab. 2014 August ; 16(8): 757–760. doi:10.1111/dom.12248.

Inhibition of Carnitine Palmitoyltransferase1b Induces Cardiac Hypertrophy and Mortality in Mice

KR Haynie¹, B Vandanmagsar¹, SE Wicks¹, J Zhang¹, and RL Mynatt^{1,*}

¹Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana, USA

Abstract

Recent reports suggest that short-term pharmacological Cpt1 inhibition improves skeletal muscle glucose tolerance and insulin sensitivity. While this appears promising for the treatment of diabetes these Cpt1 inhibitors are not specific to skeletal muscle and target multiple Cpt1 isoforms. To assess the effects of inhibiting the Cpt1b isoform we generated mice with a heart and skeletal muscle specific deletion of the Cpt1b, Cpt1b^{HM-/-}. These mice seem to develop normally with similar bodyweights as control mice. However, by 15 weeks of age the Cpt1b^{HM-/-} mice begin to die. The hearts of Cpt1b^{HM-/-} mice were 4-times the size of controls. Cpt1b^{HM-/-} mice were also subject to stress-induced seizures that accompanied an increased risk for premature mortality. Our data suggests that prolonged Cpt1b inhibition poses severe cardiac risk and emphasizes that attempts to improve insulin sensitivity by targeting Cpt1 with current inhibitors is not viable.

Introduction

Carnitine palmitoyltransferase 1 (Cpt1) is located on the outer mitochondrial membrane and is essential for transporting long chain fatty acids into the mitochondria for oxidation. Because of its central role in fatty acid oxidation, Cpt1 is an experimental and pharmacological target of studies aimed at elucidating the relationship between fat oxidation and insulin action. Recently published reports have highlighted pharmacological Cpt1 inhibitors, etomoxir and oxfenicine, as therapies for improving glucose tolerance (1, 2). Compared to high fat diet fed controls, mice administered oxfenicine display attenuated diet-induced elevations in plasma insulin and improved insulin-stimulated phosphorylation of AKT (2). In the second study, non-obese study participants administered oral etomoxir for 1 week display elevated ratios of sarcolemma to sarcoplasm GLUT4 concentrations in type 1

*Corresponding Author: Randall L. Mynatt, 6400 Perkins Road, Baton Rouge, LA 70808, 225-763-3100 (phone), 225-763-0273 (fax), Randall.Mynatt@pbrc.edu.

Author Contribution

K.R. Haynie and R.L. Mynatt prepared and wrote the manuscript. K.R. Haynie, B. Vandanmagsar and S.E. Wicks assisted with experimental design, manuscript revisions, data collection and editing. J. Zhang provided technical expertise on the experimental design of the study and the generation of the Cpt1b^{HM-/-} mouse line.

Conflict of interest details: Haynie-no competing interest Wicks-no competing interest Zhang-no competing interest Vandanmagsar-no competing interest Mynatt-no competing interest

and 2 muscle fibers, reduced HOMA-IR index, and improved insulin stimulated glucose clearance (1).

Although these findings highlight Cpt1 inhibitors as potential pharmacological therapies for skeletal muscle insulin resistance, the major negative consequence of using etomoxir as a therapeutic agent is the inhibition of Cpt1b in both cardiac and skeletal muscle and Cpt1a in liver. Chronic administration of etomoxir and inhibition of Cpt1a has the potential to cause hepatic steatosis (3). Oxfenicine is more selective towards Cpt1b, but FAO is inhibited in cardiac and skeletal muscle. Given that long chain fatty acids account for 70% of the energy utilized by the heart, Cpt1 plays a vital role in the regulation of cardiac function. This is evident in the prevalence of cardiomyopathy in patients with genetic defects in β -oxidation (4). Herein, we report the effects of Cpt1b deletion in cardiac and skeletal muscle.

Methods

Generation of muscle specific Cpt1b knockout mice

All gene targeting and chimeric mouse production was performed by the Transgenic Core at Pennington Biomedical Research Center as outlined in Figure 1A. Targeted C57BL6 ES were injected into C57BL6 blastocysts and chimeric animals were mated to C57BL6 mice to generate heterozygous offspring on a pure C57BL6 background. To generate muscle specific knockout mice for Cpt1b, Cpt1b^{lox/+} were bred to Mck-Cre recombinase mice (from Ronald Kahn, M.D.). All comparisons are from Cpt1b^{lox/lox:MckCre/+} (designated Cpt1b^{HM-/-}) and Cpt1b^{lox/lox;+/+} littermates (designated controls Cpt1b^{fl/fl}).

Animal Studies

Animal studies were conducted at Pennington Biomedical Research Center's AALAC-approved facility, and were approved by the Institutional Animal Care and Use Committee. Mice had ad libitum access to food and water and were fed a breeder chow diet, composed of 20% protein, 25% fat, 55% carbohydrate (Purina Rodent Chow no. 5015, Purina Mills, St. Louis, MO, USA). Histology and quantitative RT-PCR were performed using standard protocols.

Results

Targeted deletion of Cpt1b

Genetic ablation of Cpt1b results in embryonic lethality (5). A conditional approach for gene targeting was used to determine the physiological effects of the loss of Cpt1b activity in muscle. Mice lacking Cpt1b were generated by breeding animals carrying a floxed allele of the Cpt1b gene to mice expressing Cre recombinase under the control of the muscle creatine kinase (Mck) promoter (Figure 1A). This strategy leads to Cpt1b inactivation by deleting exons 11–15 which code for the acyl-CoA and carnitine binding sites. Analysis of Cpt1b abundance by qRT-PCR demonstrate high levels of Cpt1b expression in heart and skeletal muscle that was virtually eliminated (>98%) in the Cpt1b^{HM-/-} mice (Figure 1B). There was a compensatory increase in CPT1a in hearts of in the Cpt1b^{HM-/-} mice (Figure 1C). Body weights (Figure 1D) and body composition (not shown) in male and female mice are not

significantly different between Cpt1b^{HM-/-} and controls up to 15 weeks of age. Blood glucose levels (after a 4 hour fast) were 20% lower in Cpt1b^{HM-/-} mice, but were not statistically different from controls (Figure 1E).

Stress induced seizures

One of the first signs of abnormalities in Cpt1b^{HM-/-} mice was what appeared to be random seizures in the Cpt1b^{HM-/-} mice during routine cage changing and body weight measurements. However the seizures were temporary and the mice seem to return to normal in a few minutes. At 12 weeks of age an insulin tolerance test was attempted. One half of the Cpt1b^{HM-/-} mice experienced seizures after tail blood was collected for the zero time point before insulin injection and the test was terminated. Again seizures were temporary and the mice returned to normal activity.

Cardiac hypertrophy and increased mortality

Mouse caretakers began reporting deaths of Cpt1b^{HM-/-} mice in the breeding colony and necropsy revealed severe cardiac hypertrophy. A more systematic approach of Cpt1b^{HM-/-} and control mice between 12 and 16 weeks of age revealed a four-fold increase in heart weight in the Cpt1b^{HM-/-} mice (Figure 2B) despite similar body weights (Figure 1C). Histological evaluation demonstrated an increase in heart size in Cpt1b^{HM-/-} mice (Figure 2A) with increased myocardium thickness and left ventricular hypertrophy and increased lipid in the hearts of Cpt1b^{HM-/-} mice (Figure 2B and 2C). There was reduced mRNA for the mitochondrial marker citrate synthase, but mRNA levels for markers of complex I (Ndufs8), II (Sdhb), III (Cox5a) of the electron transport chain were normal. Heart mRNA levels for hexokinase 2 and Pdha1 subunit of pyruvate dehydrogenase complex and they were not significantly different between CPT1b KO mice and controls (Figure 2D). In the cohort of mice in which we were following body weight, both male and female Cpt1b^{HM-/-} mice began to die starting at 15–17 weeks of age. By 25 weeks of age 60% of the Cpt1b^{HM-/-} female mice and 50% of the Cpt1b^{HM-/-} male mice were deceased, possibly from congestive heart failure, and the study was terminated (Figure 2E).

Discussion

It is well established that the heart is primarily dependent on fat oxidation for energy and that this dependence increases with the development and progression of the metabolic syndrome (6). Reports that Cpt1 enzymatic activity is not altered with obesity (6) and short term (8 day) administration of etomoxir failed to increase cardiac TAG accumulation in rats (7) resulted in assumptions that Cpt1 activity had a negligible influence on cardiac function. However; other groups report cardiomyopathy in mice administered doses of etomoxir in excess of 106M (8) and for prolonged (10 days) periods of time (9). Given the variability observed between studies utilizing pharmacological Cpt1 inhibitors, genetic deletion of Cpt1b is more likely to produce consistent and reproducible conclusions.

The importance of Cpt activity in cardiac health is evident in both mice and humans with genetic deficiencies in carnitine transport (5, 10). Infantile type Cpt2 deficiency can present itself with severe cases of hypoketotic hypoglycemia which can result in cardiac damage and

premature mortality (10). Furthermore, Cpt1b^{+/-} mice display augmented cardiomyopathy when subjected to cardiac stress despite being asymptomatic under basal conditions (5). Upon stimulation of severe transverse aortic constriction (TAC), 80% of the Cpt1b^{+/-} mice develop cardiac apoptosis, congestive heart failure, and consequently premature mortality, while the survival rate is over 90% in the control animals (5). As observed with pharmaceutical Cpt1 inhibition, the severity of cardiomyopathy is likely to correlate with the degree of Cpt1 inhibition. We report that Cpt1b^{HM-/-} mice display cardiac hypertrophy, seizures, and premature mortality in the absence of mechanical cardiac stress and diet-induced obesity. Our findings highlight the importance of Cpt1b function in cardiac muscle and suggest that pharmaceutical therapies aimed at improving insulin sensitivity through Cpt1 inhibition should exclusively target skeletal muscle.

Acknowledgments

Estrellita Bermudez, Dieyun Ding, and Tamra Mendoza provided technical expertise and are thanked for their contribution to this research.

This work utilized The Pennington Biomedical Research Center Imaging Core, which is supported in part by COBRE (NIH 8 P20-GM103528) and NORC (NIH 2P30-DK072476) center grants from the National Institutes of Health. R.L.M. was supported by ADA grant # 1-10-BS-129 and NIH grants R01DK089641 and R01DK098687.

References

1. Timmers S, Nabben M, Bosma M, van Bree B, Lenaers E, van Beurden D, Schaart G, Westerterp-Plantenga MS, Langhans W, Hesselink MK, Schrauwen-Hinderling VB, Schrauwen P. Augmenting muscle diacylglycerol and triacylglycerol content by blocking fatty acid oxidation does not impede insulin sensitivity. *Proc Natl Acad Sci U S A*. 2012; 109:11711–11716. [PubMed: 22753483]
2. Keung W, Ussher JR, Jaswal JS, Raubenheimer M, Lam VH, Wagg CS, Lopaschuk GD. Inhibition of Carnitine Palmitoyltransferase-1 Activity Alleviates Insulin Resistance in Diet-Induced Obese Mice. *Diabetes*. 2012
3. Koteish A, Diehl AM. Animal models of steatosis. *Semin Liver Dis*. 2001; 21:89–104. [PubMed: 11296700]
4. Bonnet D, Martin D, Pascale De L, Villain E, Jouvet P, Rabier D, Brivet M, Saudubray JM. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation*. 1999; 100:2248–2253. [PubMed: 10577999]
5. He L, Kim T, Long Q, Liu J, Wang P, Zhou Y, Ding Y, Prasain J, Wood PA, Yang Q. Carnitine palmitoyltransferase-1b deficiency aggravates pressure overload-induced cardiac hypertrophy caused by lipotoxicity. *Circulation*. 2012; 126:1705–1716. [PubMed: 22932257]
6. Holloway GP, Snook LA, Harris RJ, Glatz JF, Luiken JJ, Bonen A. In obese Zucker rats, lipids accumulate in the heart despite normal mitochondrial content, morphology and long-chain fatty acid oxidation. *J Physiol*. 2011; 589:169–180. [PubMed: 21041527]
7. Luiken JJ, Niessen HE, Coort SL, Hoebbers N, Coumans WA, Schwenk RW, Bonen A, Glatz JF. Etomoxir-induced partial carnitine palmitoyltransferase-I (CPT-I) inhibition in vivo does not alter cardiac long-chain fatty acid uptake and oxidation rates. *Biochem J*. 2009; 419:447–455. [PubMed: 19138173]
8. Lopaschuk GD, Wall SR, Olley PM, Davies NJ. Etomoxir, a carnitine palmitoyltransferase I inhibitor, protects hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine. *Circ Res*. 1988; 63:1036–1043. [PubMed: 3197271]
9. Cabrero A, Merlos M, Laguna JC, Carrera MV. Down-regulation of acyl-CoA oxidase gene expression and increased NF-kappaB activity in etomoxir-induced cardiac hypertrophy. *J Lipid Res*. 2003; 44:388–398. [PubMed: 12576521]
10. Bonnefont JP, Demaugre F, Prip-Buus C, Saudubray JM, Brivet M, Abadi N, Thuillier L. Carnitine palmitoyltransferase deficiencies. *Mol Genet Metab*. 1999; 68:424–440. [PubMed: 10607472]

Figure 1. Targeted deletion of CPT1b. (A) Strategy used for the generation of CPT1b^{HM-/-} mice. (B) CPT1b mRNA expression (C) Bodyweight. (n= 5–8 per group) A Student's T-test was performed to determine statistical significance, * denotes p < 0.05.

Figure 2.

CPT1b^{HM-/-} mice display cardiac hypertrophy and increased mortality. (A) H&E Staining of hearts from *CPT1b^{fl/fl}* and *CPT1b^{HM-/-}* mice. (B) Heart weight (C) Sectioned hearts were stained for neutral lipid via oil red O staining and quantified (D) Quantitative RT-PCR of mitochondrial and glucose metabolism enzymes (E) Survival curve. (n= 5–8 per group) A Student's T-test was performed to determine statistical significance, * denotes $p < 0.05$.