Mitochondrial Fatty Acid Oxidation Deficiency Exacerbates Diet-Induced Fatty Liver Disease

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BACKGROUND

Fatty liver disease (FLD), often caused by high-fat diets, is a major health concern. This study investigates:

- *The role of fatty acid oxidative metabolism in FLD*
- *Potential of other fatty acid metabolism pathways as therapeutic targets for FLD*

HYPOTHESIS

We hypothesize that mitochondrial fatty acid oxidation is crucial in FLD pathogenesis. Knocking out the CACT gene in mice will result in more severe FLD progression on a high-fat diet compared to controls, with consistent molecular markers of mitochondrial and peroxisomal dysfunction.

MATERIALS & METHODS

Gene Knockout

- *CACT knockout mice were compared to wild-type C57BL/6J controls.*

Fatty Liver Diet Regimen

Mice were divided into four groups:

- *Control genotype / control diet (Control) (n=5)*
- *Control genotype / fatty liver diet (Control FLD) (n=4)*
- *CACT knockout / control diet (CactLiv-/-) (n=4)*
- *CACT knockout / fatty liver diet (CactLiv-/-) (n=5)*

Control diet: standard chow (10 kcal% fat)
Fatty liver diet: high-fat (60kcal% fat) for 7 days
Liver weight measured post-diet

RT-PCR Analysis

- *Quantitative real-time PCR with SYBR Green on QuantStudio 6 Flex*
- *Primers for mitochondrial biosynthesis, gluconeogenesis, fatty acid oxidation, ketogenesis, triacylglycerol synthesis*
- *B2M as housekeeping gene*
- *Samples run in duplicate with melt curve analysis for specificity*

Data Analysis

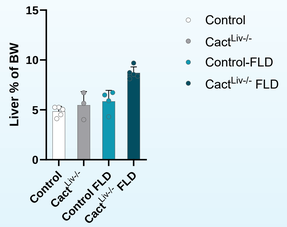
Relative gene expression: 2^-ΔΔCt method
Statistical analysis: GraphPad Prism, $p < 0.05$ considered significant

RESULTS

Liver weight:

- *Increased in CactLiv-/- FLD group*

Fig. 1: Percentage Liver of Mouse Total Body Weight (BW)



Gene expression changes:

CactLiv-/- mice:

- *↑ Gluconeogenesis, mitochondrial/peroxisomal fatty acid oxidation, carnitine synthesis, ketogenesis*

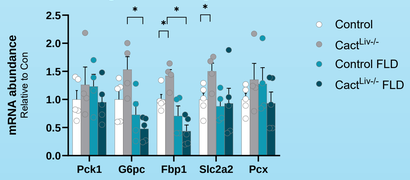
CactLiv-/- FLD mice:

- *↑ Pparg (lipogenesis)*
- *↓ Gluconeogenesis, carnitine synthesis, most mitochondrial fatty acid oxidation genes (except cpt1a)*

Control FLD mice:

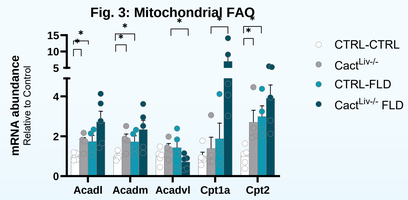
- *↑ Mitochondrial fatty acid oxidation, ketogenesis, systemic metabolism, peroxisomal fatty acid oxidation*

Fig. 2: Gluconeogenesis



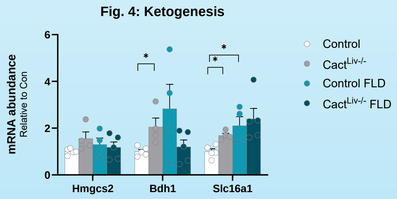
- Compared to Control, CactLiv-/- mice show upregulation of pcx(p=0.030), and fbp1 (p=0.021). Yet, when compared to CactLiv-/- mice, CactLiv-/- FLD mice show downregulation of G6pc (p-value=0.002) and Fbp1 (p-value=0.0004).

Fig. 3: Mitochondrial FAO



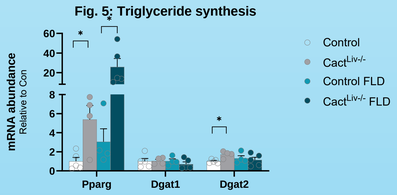
- Acadl (p=0.012), Cpt2 (p=0.054), and Acadm (p=0.018) present with increased abundance compared to control. Control shows moderate increase in expression of cpt2 (0.007) under FLD diet. CactLiv-/- shows considerable upregulation of cpt1a (p=0.051) and moderate decrease in acadvl (p=0.046).

Fig. 4: Ketogenesis



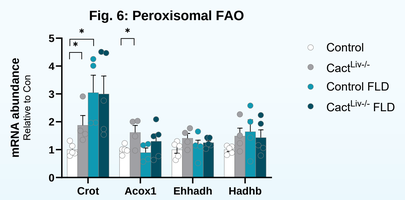
- CactLiv-/- samples exhibit an increase of abundance for Scl16a1 (p=0.003), and bdh1 (p=0.020) when compared to Control. Slc16a1 also increased in Control FLD (p=0.018).

Fig. 5: Triglyceride synthesis



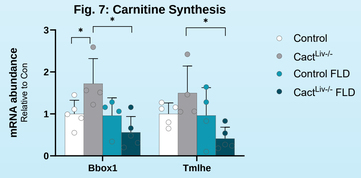
- Pparg (p=0.015) and Dgat2 (p=0.006) are seen with increased abundance in CactLiv-/- mice. Pparg abundance is also seen to be greater in CactLiv-/- FLD mice than Control FLD mice.

Fig. 6: Peroxisomal FAO



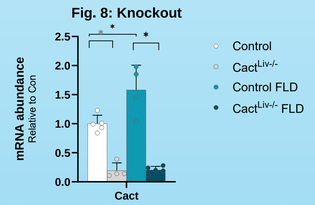
- CactLiv-/- mice exhibit increased abundance of Crot (p=0.032) and acox1 (p=0.034) compared to Control. Crot is also seen in higher abundance in Control mice fed fatty liver inducing diet when compared to control diet (p=0.008).

Fig. 7: Carnitine Synthesis



- *Bbox1 (p=0.05) is seen in higher abundance in CactLiv-/- mice vs. Control. Yet, there is a significant decrease in abundance in Bbox1 (p=0.009) and Tmlhe (p=0.011) in CactLiv-/- mice fed a fatty liver inducing diet, compared to CactLiv-/- on a control diet.*

Fig. 8: Knockout



- *Gene Analysis shows that there is significant absence of Cact in knockout mice in Control vs CactLiv-/- mice under control diet (p=0.00006) and FLD diet (p=0.0002). There is also a significant increase in Cact (p=0.02) in Control mice fed the FLD diet compared to those on control diet.*

CONCLUSION/DISCUSSION

These findings suggest that mitochondrial fatty acid oxidation is crucial in protecting against FLD. CACT knockout impairs the liver's adaptive response to a high-fat diet, and alternative fatty acid oxidation pathways may be activated as compensatory mechanisms. Further research is needed to explore potential therapeutic targets based on these findings

Next Steps:

- *Conduct a more comprehensive analysis of gene expression changes, possibly using RNA sequencing to identify additional pathways involved in the compensatory response to CACT knockout.*
- *Perform metabolomic studies to better understand the alterations in lipid metabolism and identify potential biomarkers.*
- *Investigate the effects of pharmacological interventions that target the upregulated pathways identified in this study, such as peroxisomal fatty acid oxidation, in CACT knockout mice.*

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