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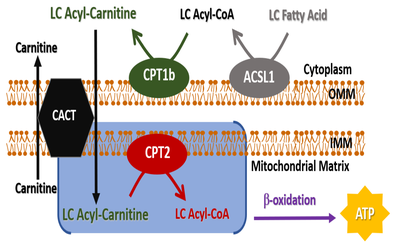
Determining the Effects of Impaired Muscle Fatty Acid Oxidation on Liver Metabolism

Introduction

Skeletal muscle plays an extremely critical role in maintaining glucose homeostasis, and is responsible for the majority of glucose uptake for energy production. Carnitine palmitoyltransferase II (CPT2) is an irreplaceable enzyme for oxidation of long-chain fatty acids in muscle, converting acylcarnitine into acyl-CoA within the mitochondrial matrix to allow for β-oxidation. Defects in CPT2 impair mitochondrial β-oxidation and result in the buildup of acylcarnitines within the skeletal muscle which are predicted to impair glucose metabolism.



Methods

Real-Time PCR was utilized to determine the abundance of codifying RNA (mRNA) involved in mitochondrial β-oxidation, gluconeogenesis, and ketogenesis in liver samples from 4 experimental groups of 6 mice each: 1) control mice on low fat diet ($Cpt2^{Sk+/+}$ LF) 2) control mice on a 60% high-fat diet ($Cpt2^{Sk+/+}$ HF) 3) knockout mice on LF diet ($Cpt2^{Sk-/-}$ LF) 4) knockout mice on HF diet ($Cpt2^{Sk-/-}$ HF). To aid the metabolic flux impaired by acylcarnitine accumulation, free carnitine was supplemented to another set of mice with the same grouping as above, and each group consisted of 3 mice. Gene expression was normalized to the housekeeping enzyme Beta-2-Microglobulin (B2M).

1. $Cpt2Sk^{-/-}$ Mouse Model

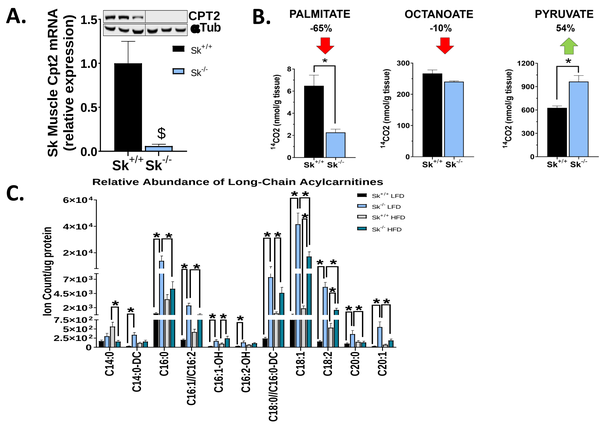


Figure 1. To assess the specific effects of Cpt2-deficiency on muscle and whole-body physiology, we generated a skeletal muscle specific CPT2-knockout mouse model ($Cpt2^{Sk-/-}$). Deletion of skeletal muscle Cpt2 was confirmed in the soleus muscle both in the proteome (Western Blot) and transcriptome (RT-PCR) **(A)**. Compared to controls, the skeletal muscles of $Cpt2^{Sk-/-}$ mice have an approximately 65% decrease in fatty acid oxidation of the LC-fatty acid palmitate and a resulting increase in pyruvate oxidation to mitigate energy demands **(B).** They also accumulate up to 200-fold more acylcarnitines in muscle homogenates **(C)**. Data is shown as average ± SEM. *p<0.05

2. Hepatic Synthesis of Alternative Substrates

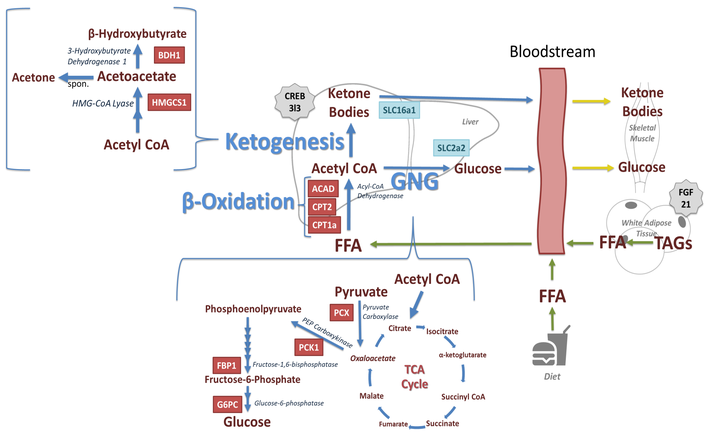


Figure 2. Because whole-body glucose homeostasis is highly regulated by liver metabolism, 14 genes in three main hepatic pathways (GNG: gluconeogenesis, KG: ketogenesis and β-Oxidation of fatty acids) were assessed. Enzyme-coding genes are indicated as red boxes in the corresponding steps in the pathways. Metabolite transporters were also evaluated and are indicated as light-blue boxes. Master regulators are depicted as grey 10-point stars.

3. Liver adaptations to muscle-specific CPT2 deficiency

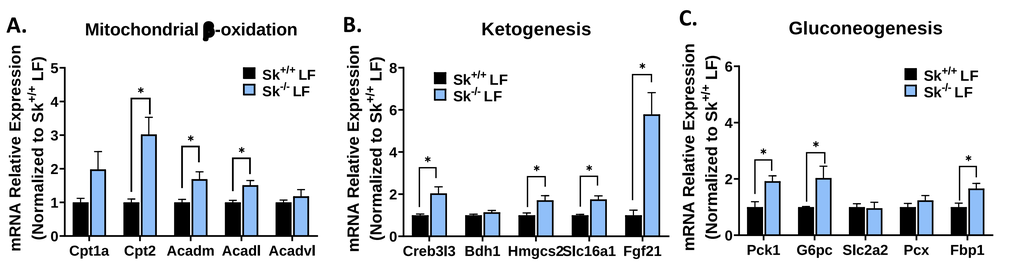


Figure 3. Hepatic adaptations to skeletal muscle-specific CPT2 deficiency involves upregulation of metabolic pathways aiming to produce alternative fuel sources.

Upon abolishment of fatty acid oxidation in the skeletal muscle, the liver of Cpt2 knockout mice ($Sk^{-/-}$ LF) significantly upregulated the expression of enzymes involved in the production of ketone bodies **(B)** and glucose **(C)** that can be utilized by peripheral tissues like muscles as alternative fuel sources. Ketogenesis is dependent on high rates of FAO, thus genes of FAO are upregulated in knockout (**A).** Data is shown as average ± SEM. *p<0.05.

4. Liver adaptations under high fat feeding

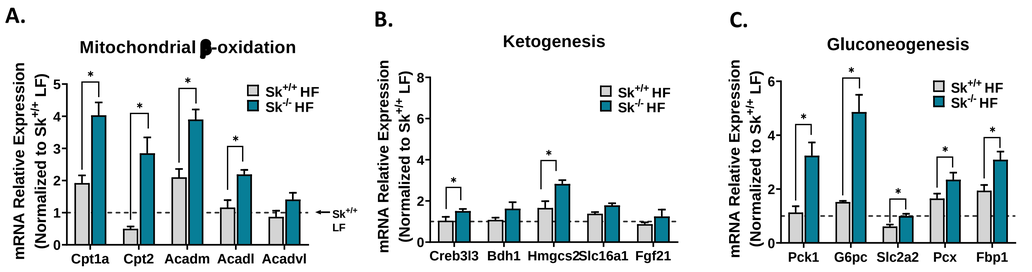


Figure 4. Under a high fat diet, hepatic adaptations to skeletal muscle-specific CPT2 deficiency involves increased upregulation of metabolic pathways aiming to produce alternative fuel sources.

Due to very low glucose availability from the diet coupled with the inhibition of fatty acid oxidation in the skeletal muscle, Cpt2 knockout mice ($Sk^{-/-}$ HF) are even more dependent on alternate sources of energy, shown by the significant upregulation of genes involved in gluconeogenesis even compared to $Sk^{-/-}$ LF. **(C)**. The high fat diet naturally upregulates mitochondrial β-oxidation **(A)**, also enabling the upregulation of the production of ketone bodies as an alternative energy source **(B)**.

5. Effects of Carnitine Supplementation

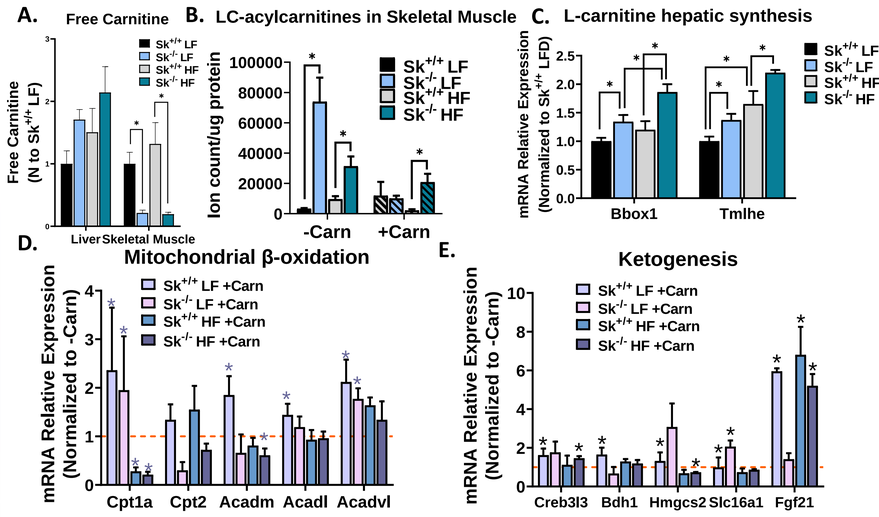


Figure 5. Exogenous L-carnitine supplementation obliterated the genotype-specific upregulation of fatty acid oxidation, gluconeogenesis, and ketogenic genes in liver. Muscle–specific Cpt2 knockout mice have significantly lower levels of free, available carnitine in their skeletal muscles due to high accumulation of LC-acyl-carnitines **(A, B)**. The muscle carnitine deficit triggers the upregulation of genes involved in hepatic carnitine synthesis like Gamma-Butyrobetaine Hydroxylase 1 (Bbox1) and Trimethyllysine Hydroxylase (Tmlhe) in Cpt2 knockout mice **(C)**.

In order to attempt restoring metabolic flux, free carnitine was supplemented to the drinking water of mice for 16 weeks and liver gene expression for FAO **(D),** KG **(E)** and GNG **(F)** was assessed again. Data was normalized to the respective Non-Carnitine groups (dashed orange line). Data is shown as average ± SEM. *p<0.05.

Conclusions

- Our novel $Cpt2^{Sk-/-}$ mouse model can modify expression of genes involved in hepatic biosynthetic pathways in order to provide alternative substrates to the mtFAO–deficient skeletal muscle, demonstrating the role of multi-organ physiological cross talk as a regulator of metabolic homeostasis.
- Free carnitine levels in $Cpt2^{Sk-/-}$ mice are significantly reduced in the skeletal muscle and hepatic synthesis of L-carnitine is consequently upregulated.
- Further supplementation of L-Carnitine in the drinking water aids the flux of metabolic intermediaries thus eliminating the genotypic upregulation of these pathways in knockout.

References

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