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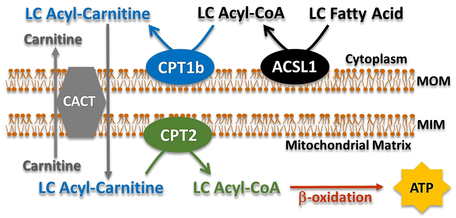
Loss of Carnitine Palmitoyltransferase-2 in Skeletal Muscle results in Muscle Remodeling and Tissue-specific Sensitivity to Insulin.

Introduction

The skeletal muscle is responsible for ~90% of the insulin-stimulated glucose uptake, thus its susceptibility to insulin resistance is critical for glucose homeostasis. Perturbed muscle metabolism of fatty acids is a common feature of overfeeding and obesity and it is associated with impaired insulin action. However, the role of muscle fatty acid oxidation (FAO) in the etiology of obesity-related insulin resistance remains debated. **We hypothesize that excessive muscle fatty acid oxidation is causal in diet-induced obesity and insulin resistance. To test this we have generated a novel mouse model with impaired mitochondrial long-chain fatty acid oxidation by deleting Carnitine Palmitoyltransferase-2 (Cpt2Sk$^{-/-}$) specifically in the skeletal muscle.**

Carnitine Palmitoyltransferase-2 (CPT2) is a ubiquitous enzyme located in the inner mitochondrial membrane. It is a key component of the carnitine palmitoyltransferase system which imports long-chain fatty acids (LCFAs) into the mitochondria for further beta-oxidation[1]. Because there is only one known isoform of CPT2, and because LCFAs are the major fraction of FAs supplied to target tissues, **CPT2 deficiency significantly impacts fatty acid-derived energy production[2,3].**



1. CPT2 Deficiency in the Skeletal Muscle Prevents High Fat Diet-induced Obesity

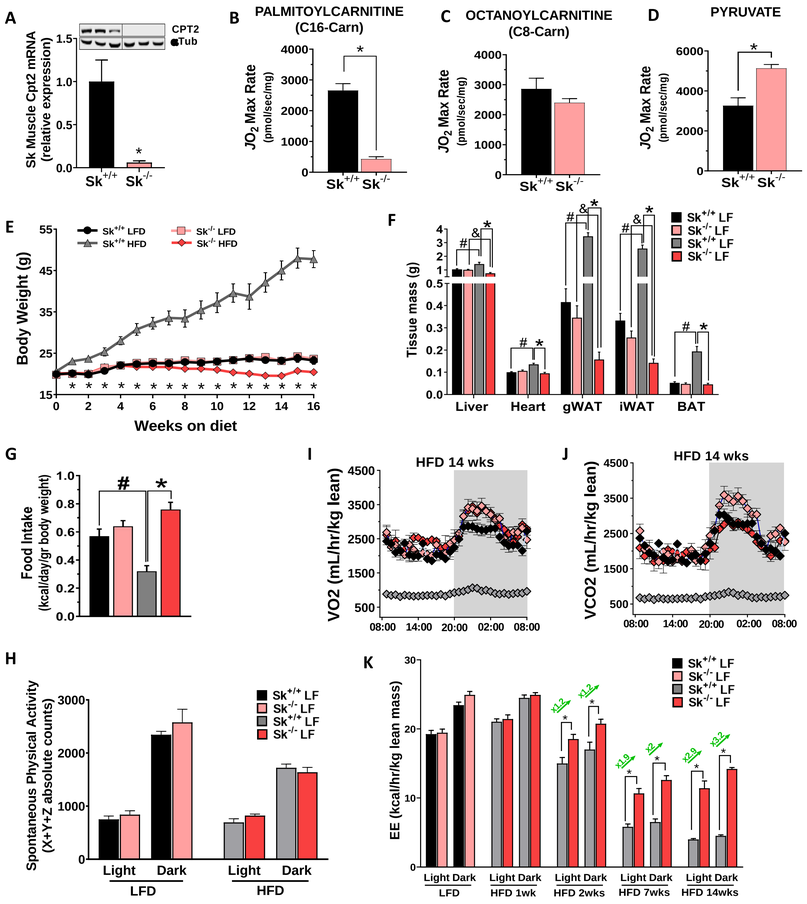


Figure 1. CPT2 deficiency in the skeletal muscle prevents high fat diet-induced adiposity.

CPT2-targeted mice were crossed with human alpha-skeletal actin (ACTA1)-Cre mice to obtain CPT2 knock out (Cpt2Sk$^{-/-}$) and control (Cpt2Sk$^{+/+}$) mice. Cpt2 deletion in skeletal muscle was at the gene and protein level (upper panel) **(A).** Substrate oxidation capacity was measured in isolated muscle mitochondria for long-chain **(B)** and medium-chain fatty acids **(C)** and for pyruvate **(D)**. Then Cpt2Sk$^{-/-}$ female mice and control littermates were fed either a high-fat (HF, 60% kcal/gr fat) or a low-fat diet (LF, 16% kcal/gr fat) *ad libitum* for 16 weeks. Body weight (BW) **(E)** and liver, heart, gonadal (gWAT) and inguinal (iWAT) white adipose tissue and brown adipose tissue (BAT) **(F)** were recorded at the end of the intervention. Open circuit indirect calorimetry was performed after 14 weeks of low-fat and high-fat diet intervention allowing us to calculate food intake (G), home-cage physical activity (H), O2 consumption (I), CO2 production (J) and energy expenditure (K). Data values are presented as the mean ± S.E.M. n=3-5 for panels A to D; n=11-14 for E and F and n=6 to 10 for G to K. # p<0.05 Sk$^{+/+}$ HF vs. Sk$^{+/+}$ LF; $ p<0.05 Sk$^{-/-}$ LF vs. Sk$^{+/+}$ LF; * p<0.05 Sk$^{-/-}$ HF vs. Sk$^{+/+}$ HF; & p<0.05 Sk$^{-/-}$ HF vs. Sk$^{-/-}$ LF.

2. CPT2 Deficiency in the Skeletal Muscle Differentially Modulates Insulin Sensitivity

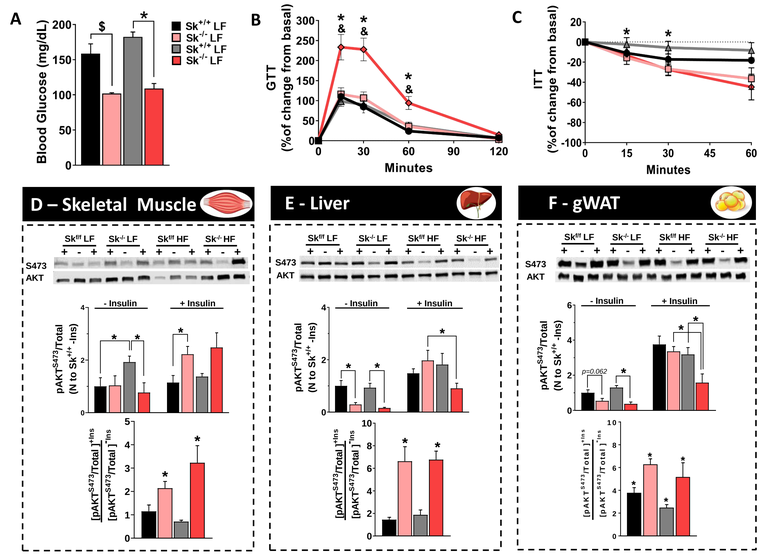


Figure 2. Muscle-specific CPT2 deficiency differentially modulates insulin sensitivity at tissue and whole-body level.

Fasting blood glucose were determined in both Cpt2Sk-/- or Cpt2Skf/f mice after 14 weeks on a high fat (HF) or a low fat diet (LF) **(A)** . Glucose Tolerance Test (GTT) was performed at week 15 **(B)** and Insulin Tolerance Test at week 16 on the diet **(C).** Tissue-specific insulin sensitivity was assessed via Akt activation by Western Blot in TA muscle **(D)**, liver **(E)** and gWAT **(F).** Data values are presented as the mean ± S.E.M. n=12-15 for panels A, B and C; n=6 for panels D, E, F; # p<0.05 Skf/f HF versus Skf/f LF; $ p<0.05 Sk-/- LF versus Skf/f LF; * p<0.05 Sk-/- HF versus Skf/f HF; & p<0.05 Sk-/- HF versus Sk-/- LF.

3. CPT2-deficient skeletal muscles have a unique lipid profile

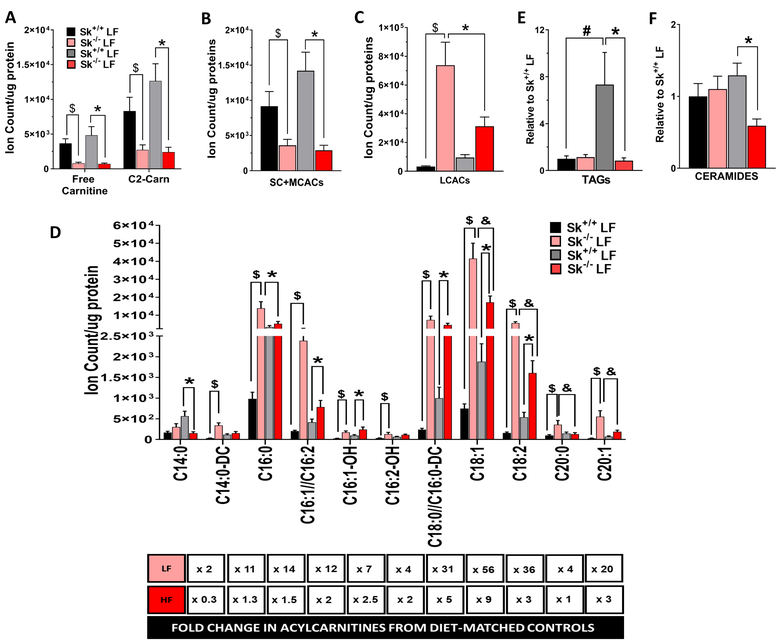


Figure 3. Muscle-specific CPT2 deficient mice show increased long-chain acylcarnitines in TA muscle with no accumulation of other lipotoxic species.

Skeletal muscle lipid profile was characterized in Cpt2Sk-/- or Cpt2Skf/f mice after 16 weeks on a high fat (HF) or a low fat diet (LF). The following species were measured in the TA muscle: **(A)** Free carnitine, **(B)** short- and medium-chain acylcarnitines (SC+MCACs), **(C)** long-chain acylcarnitines (LCACs), **(E)** ceramides, and **(F)** TAGs. Panel (**D**) display specific LCACs and their relative abundance when compared to control groups (table). Data values are presented as the mean ± S.E.M. n=5-6

4. CPT2 Deficiency Triggers Skeletal Muscle Remodeling

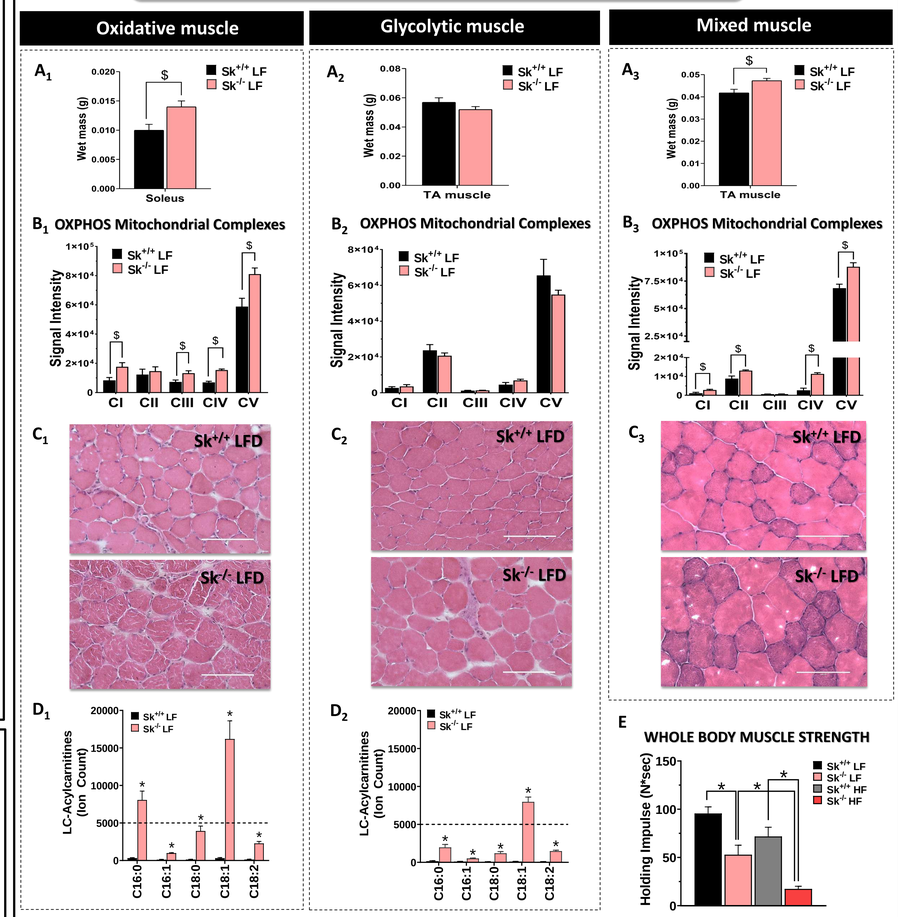


Figure 4. CPT2 deficiency in the skeletal muscle increases muscle mass and mitochondrial content

Soleus (SOL), Tibialis Anterior (TA) and Extensor Digitorum Longus (EDL) muscles from mice on Low Fat Diet (LFD) were collected and weighted **($A_{1,2,3}$)**. Electron transport chain subunits (OxPhos complexes CI to CV) were visualized by western blot and quantitated in SOL and TA homogenates **($B_{1,2,3}$).** Histological sections of SOL, EDL and TA muscle were stained with H&E to reveal tissue general structure **($C_{1,2,3}$).** Amount of long-chain acylcarnitines were measured as described in panel 3 **(D)**. Muscle strength and endurance was assessed using the wire hang test and the latency to fall was recorded **(E)**. Data values are presented as the mean ± S.E.M. n=10-14 for panels A and E; n=3-6 for panels B to D; $ p<0.05 Sk$^{-/-}$LF versus Skf/fLF.

Conclusions

- We have generated a novel mitochondrial-FAO-deficient mouse model (Cpt2Sk$^{-/-}$) that is **severely resistant to high-fat diet (HFD)-induced obesity.** After 16 weeks of high-fat feeding, Cpt2Sk$^{-/-}$ mice have **94% reduction in white adipose tissue mass,** have overall **preserved muscle mass** and are protected against HFD-induced hepato- and cardiomegaly.
- Indirect calorimetry shows that HFD-fed Cpt2Sk$^{-/-}$ mice have increased Energy Expenditure which might contribute to the observed leanness.
- Cpt2Sk$^{-/-}$ mice have **lower basal glycaemia** and **differential insulin sensitivity** across different tissues. While liver and adipose tissue of Cpt2 knockout mice display a profound Akt hypophosphorylation under basal conditions, skeletal muscle retains normal phos-Akt levels, suggesting that other peripheral tissues might be sparing glucose for skeletal muscle usage.
- CPT2 deficient **muscles** undergo **significant remodeling** in a muscle type dependent manner. Muscles with high content of oxidative fibers, like Soleus, seem to be more susceptible to long-chain acylcarnitine accumulation, increased mitochondrial content and altered substrate/fuel preferences. Despite these adaptive attempts, fatty acid oxidation deficient muscles have compromised function as evidenced by decreased general muscle force.

References and Acknowledgements

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