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Supplemental Information

**Loss of Muscle Carnitine Palmitoyltransferase 2
Prevents Diet-Induced Obesity and Insulin Resistance
despite Long-Chain Acylcarnitine Accumulation**

Andrea S. Pereyra, Arvind Rajan, Christina R. Ferreira, and Jessica M. Ellis

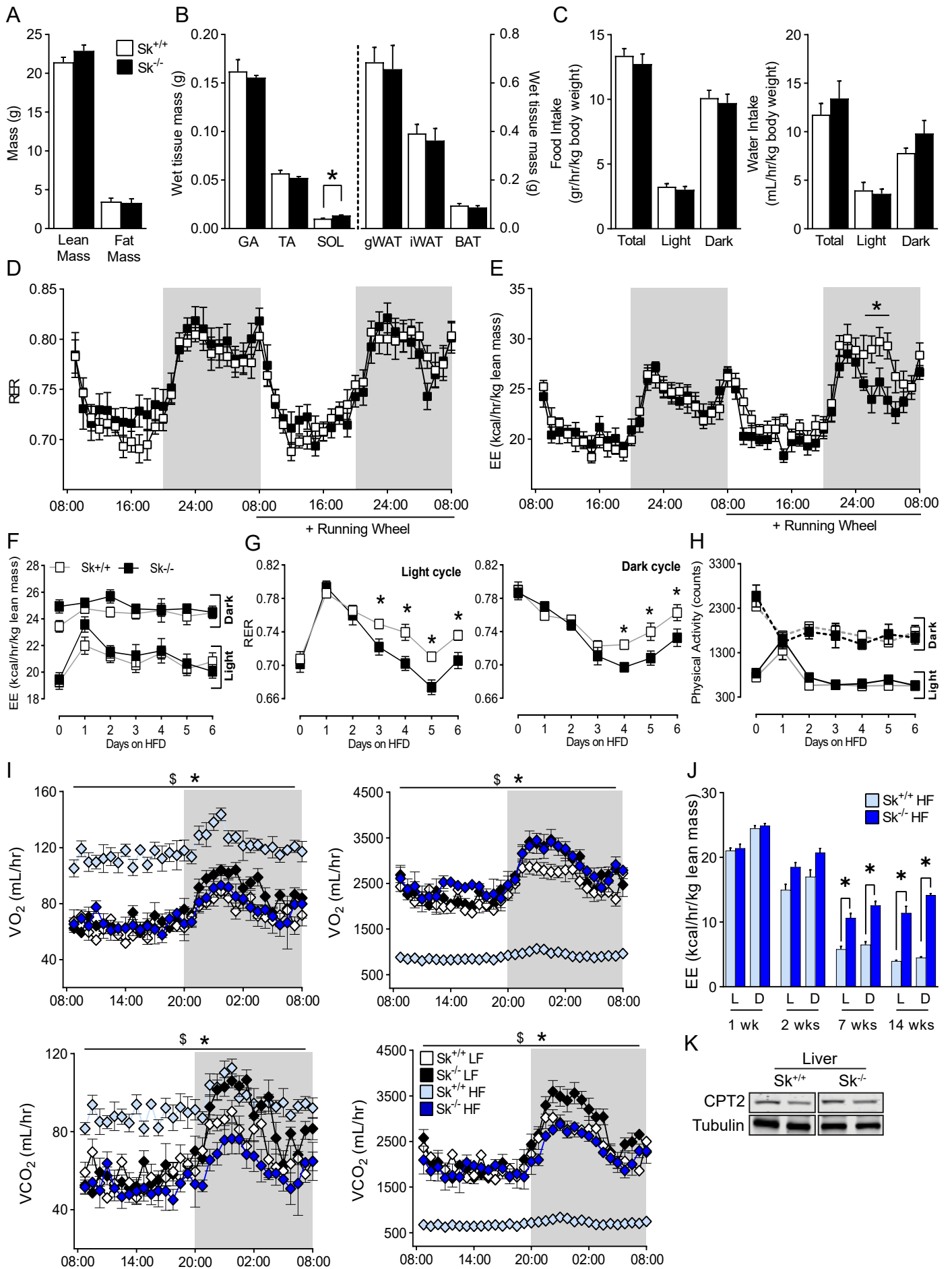


Figure S1. Metabolic parameters in Cpt2Sk^{-/-} mice and CPT2 expression in liver. Related to Figure 1.

- (A) Whole-body composition of Cpt2Sk^{+/+} and Cpt2Sk^{-/-} male mice at 16 weeks of age (n=7-12).
- (B) Wet mass of gastrocnemius (GA), tibialis anterior (TA), soleus (SOL) muscle and gonadal- (gWAT), inguinal (iWAT), and brown-adipose tissue (BAT), males, 18 weeks of age (n=7-10).
- (C) Food and water intake registered during indirect calorimetry, males, 12 weeks of age (n=8).
- (D) Resting energy requirements (RER) during light and dark cycles, with and without voluntary physical activity as measured by indirect calorimetry, males, 12 weeks (n=8).
- (E) Energy expenditure (EE) during light and dark cycles, with and without voluntary physical activity as measured by indirect calorimetry, males, 12 weeks (n=8).
- (F) EE during low-fat to high-fat diet transition, males, 12 weeks (n=4-5).
- (G) RER during low-fat to high-fat diet transition on light (left) and dark (right) cycles males, 12 weeks (n=4-5).
- (H) Spontaneous home cage activity during low-fat to high-fat diet transition, males, 12 weeks of age (n=4-5).
- (I) Oxygen consumption (VO₂; top) and carbon dioxide production (VCO₂; bottom) at 14 weeks of LF or HF diet feeding, non-normalized (left) and normalized (right) to lean body mass, females (n=3-5).
- (J) Energy expenditure (EE) at several time points during HF diet feeding, females (n=3-10).
- (K) Expression of CPT2 protein in liver of Cpt2Sk^{+/+} and Cpt2Sk^{-/-} female mice at 26 weeks of age (n=5).

Data is presented as mean±SEM. *P<0.05 determined by Student's t-test for Cpt2Sk^{+/+} HF diet vs. Cpt2Sk^{-/-} HF diet; \$P<0.05 for Cpt2Sk^{+/+} LF diet vs. Cpt2Sk^{+/+} HF diet.

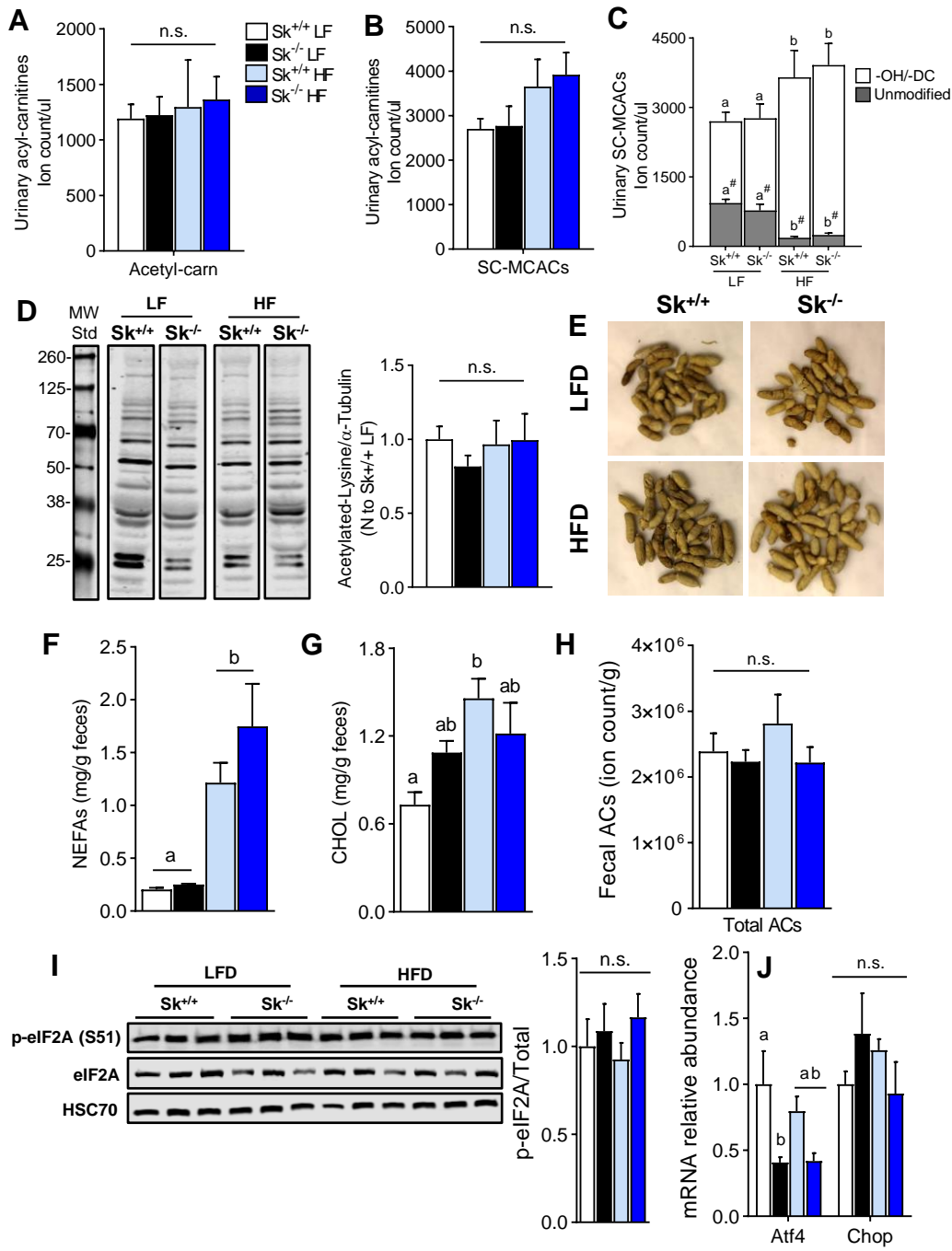


Figure S2. Loss of muscle CPT2 does not affect urinary acylcarnitine excretion nor protein acetylation nor integrated stress response but increases fecal lipid content during high-fat diet feeding. Related to Figure 2.

(A) Urinary levels of acetyl-carnitine before and after a 10-day long HF diet feeding, males (n=6).

(B) Urinary levels of short- and medium-chain acylcarnitines before and after a 10-day long HF diet feeding, males (n=6).

(C) Urinary levels of unmodified, hydroxylated (-OH) and dicarboxylated (-DC) short- and medium-chain acylcarnitines before and after a 10-day long HF diet feeding, males (n=6).

(D) Acetylated-lysine in skeletal muscle homogenates, males on low-fat diet, 12 weeks (n=6).

(E) Macroscopic aspect of feces after 8 weeks of LF or HF diet feeding, males.

(F) Fecal levels of non-esterified fatty acids (NEFAs) after 8 weeks of LF or HF diet feeding, males (n=5).

(G) Fecal levels of cholesterol esters (CHOL) after 8 weeks of LF or HF diet feeding, males (n=5).

(H) Fecal levels of acyl-carnitines (ACs) after 8 weeks of LF or HF diet feeding, males (n=5).

(I) Phosphorylation status of Eukaryotic Translation Initiation Factor 2A (eIF2A) at serine residue 51 in TA muscle, females, LF and HF diet for 16 weeks. Data is normalized to control mice on low-fat diet (n=6).

(J) Muscle mRNA abundance of markers of the mitochondrial stress response, females, LF and HF diet for 16 weeks. Data is normalized to control mice on low-fat diet (n=6).

Data is presented as mean \pm SEM. Statistical analysis by 2-way ANOVA. Means depicting a different letter indicate significant differences between groups ($P<0.05$). #indicates multiple comparisons for unmodified acylcarnitines in (C).

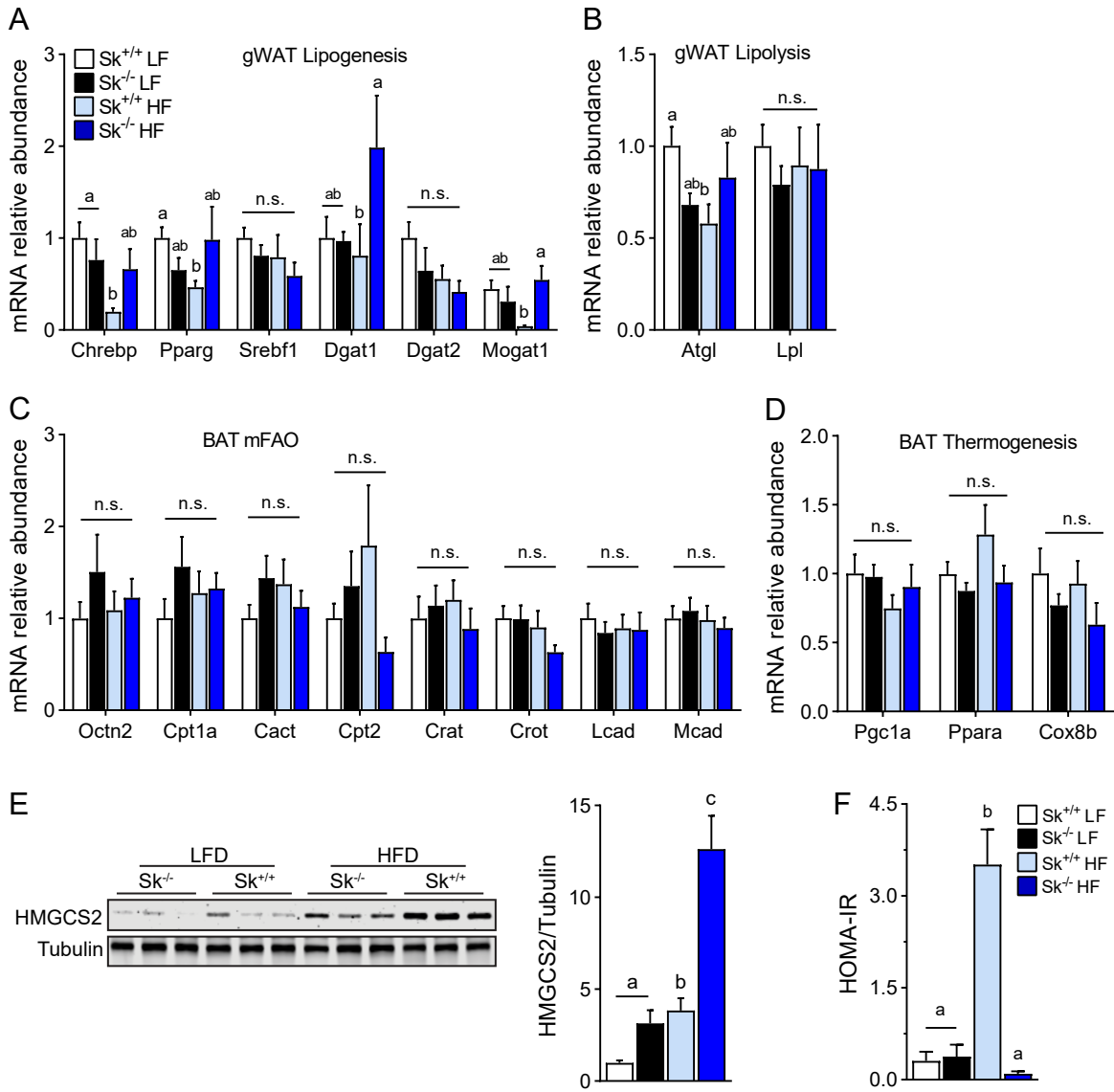


Figure S3. Expression of lipid metabolism genes in gWAT and BAT and ketogenesis enzymes in liver. Related to Figure 2, 3 and 4.

(A-B) gWAT mRNA abundance of markers of lipid metabolism, females, LF and HF diet for 16 weeks. Data is normalized to control mice on low-fat diet (n=6).

(C-D) BAT mRNA abundance of markers of fatty acid oxidation and thermogenesis, females, LF and HF diet for 16 weeks. Data is normalized to control mice on low-fat diet (n=6).

(E) Liver expression of ketogenesis enzyme HMGCS2, females, LF and HF diet for 16 weeks. Data is normalized to control mice on low-fat diet (n=6).

(F) Homeostatic model assessment of insulin resistance (HOMA-IR) index, males, LF and HF diet for 8 weeks (n=6).

Data is presented as mean±SEM. Statistical analysis by 2-way ANOVA. Means depicting a different letter indicate significant differences between groups (P<0.05).

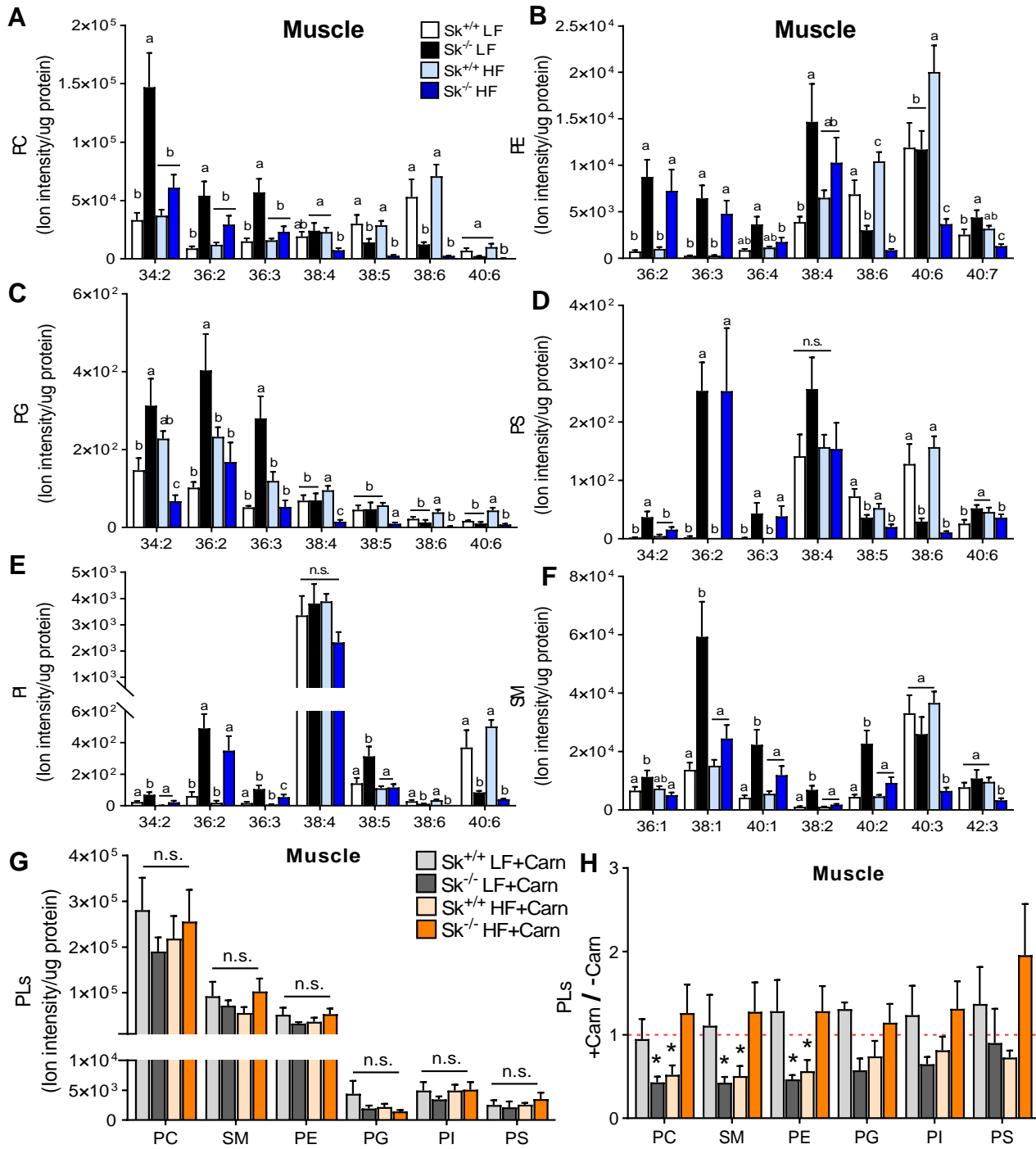


Figure S4. Loss of muscle CPT2 alters phospholipid acyl-chain composition. Effects of L-carnitine supplementation. Related to Figure 5.

(A-F) Relative abundance of phosphatidyl-choline (PC), - ethanolamine (PE), -glycerol (PG), -serine (PS) and inositol (PI) and, sphingomyelin (SM) respectively, TA muscle, females, LF and HF diet for 16 weeks (n=6).

(G) Relative abundance of phosphatidyl-choline (PC), - ethanolamine (PE), -glycerol (PG), -serine (PS) and inositol (PI) and, sphingomyelin (SM) in TA muscle of female mice, LF and HF diet with L-carnitine supplementation for 16 weeks (n=3-4).

(H) Fold change of phospholipids presented in S4A from no carnitine equivalent samples (n=6 for –Carn and n=3-4 for +Carn).

Data is presented as mean±SEM. Statistical analysis by 2-way ANOVA. Means depicting a different letter indicate significant differences between groups (P<0.05). *Depicts p<0.05 between +Carn versus –Carn for each genotype and diet determined by Student's t-test.

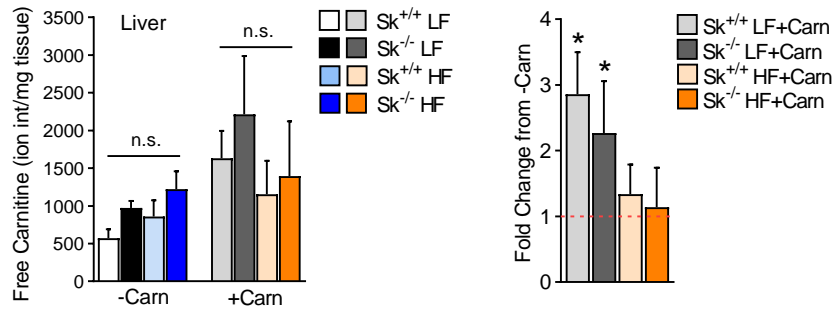
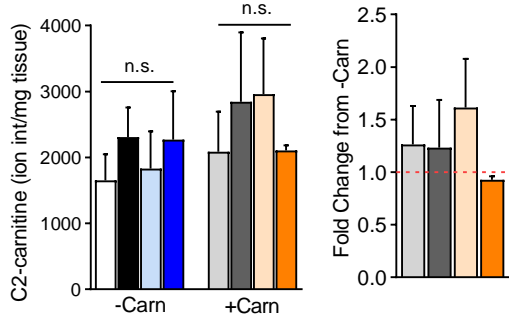
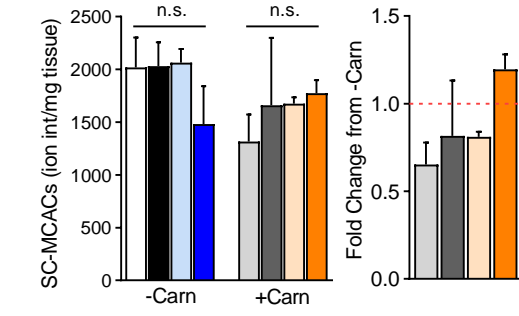
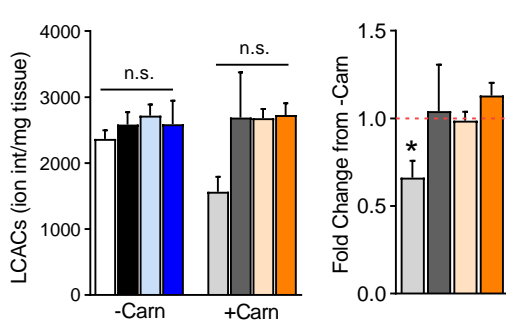
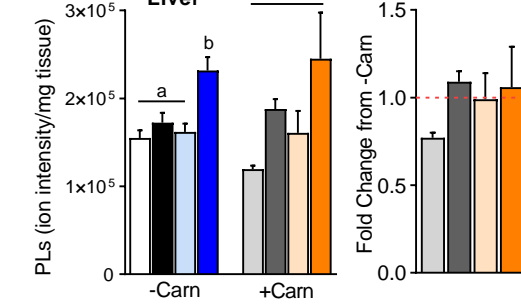
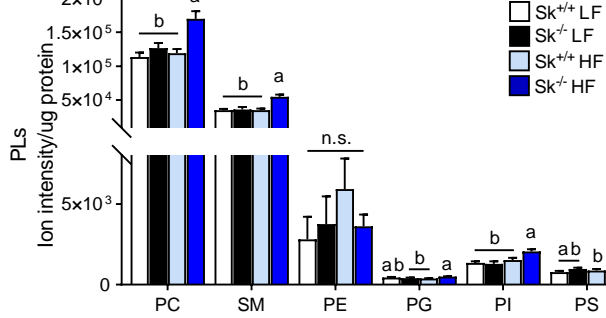
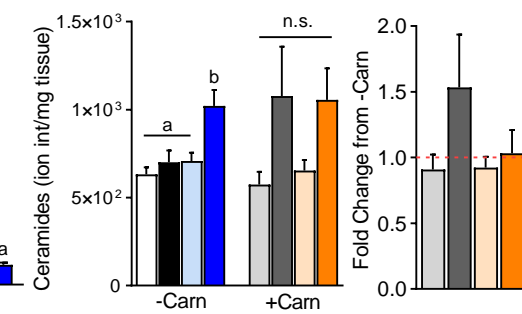
A**B****C****D****E****F****G**

Figure S5. Effects of muscle-specific CPT2 deficiency in hepatic lipid profile. Related to Figure 6.

(A-D) Relative abundance of free carnitine, acetyl-carnitine, short- and medium-chain acylcarnitines and long-chain acylcarnitines respectively in liver, females, LF and HF diet, with and without L-carnitine (n=6 for –Carn and n=3-4 for +Carn).

(E) Relative abundance of total phospholipids in liver, females, LF and HF diet, with and without L-carnitine (n=6 for –Carn and n=3-4 for +Carn).

(F) Relative abundance of phosphatidyl-choline (PC), - ethanolamine (PE), -glycerol (PG), -serine (PS) and inositol (PI) and, sphingomyelin (SM) in liver of female mice, LF and HF diet for 16 weeks (n=6).

(G) Relative abundance of ceramides in liver, females, LF and HF diet, with and without L-carnitine (n=6 for –Carn and n=3-4 for +Carn).

Data is presented as mean±SEM. Statistical analysis by 2-way ANOVA. Means depicting a different letter indicate significant differences between groups ($P<0.05$). *Depicts $p<0.05$ between +Carn versus –Carn for each genotype and diet determined by Student's t-test.