Supplemental Information

Fatty acid oxidation by the osteoblast is required for normal bone acquisition in a sex and diet-dependent manner.

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Supplemental Experimental Procedures

Animal Models

$Atgl^{lox/lox}$ mice (1) were kindly provided by Dr. Erin Kershaw and crossed with Oc-Cre mice (2) to generate osteoblast-specific mutants. Skeletal phenotyping and osteoblast cultures were completed as described in the main text.

Acylcarnitine measurements

Cellular acylcarnitine levels were quantified in frozen cell pellets containing approximately $1x10^6$ cells with modification of previously described methods (3-5). In brief, cell pellets were resuspended in 100µl of a methanol solution containing internal standards for acylcarnitines (NSK B, Cambridge Isotopes) and sonicated for 10 min before centrifugation for 4 min at 13,000 rpm at 4°C. The liquid phase was collected and dried under LN2, resuspended in 3N HCl in n-butanol, incubated at 65°C for 15 min, and then redried under nitrogen. Butylated acylcarnitines were then reconstituted in 100 µl of the mobile phase of acetonitrile/water/formic acid (H2O:CH3CN:HCOOH; 80:19.9:0.1 v/v%). Acylcarnitines were then analyzed on an API 3200 (AB SCIEX) operated in positive ion mode employing a precursor ion scan for m/z 85, which is generated as a characteristic product ion of butyl ester of acylcarnitine species. Quantitation of acylcarnitines was achieved by the Chemoview (AB SCIEX) application. All samples are reported as pmol/mg of protein.

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Supplemental Figure Legends

Supplementary Table I. Primers used in genotyping and qPCR analysis of mRNA levels

Supplementary Table II. Cellular Acylcarnitine analysis in control and ΔCpt2 osteoblast cultures.

Supplementary Figure 1. Expression of Cpt2 in metabolic tissues (A and B) qPCR analysis of Cpt2 mRNA levels in tissue isolated from male (A) and female (B) control and Cpt2 mutant mice $n \geq 5$ mice per genotype. All data are shown as mean ± SEM.

Supplementary Figure 2. Osteoblast-specific Atgl mutants (ΔAtgl) have normal trabecular bone volume.

(A) Body weight was assessed weekly from 3 to 12 weeks of age. (B) Trabecular bone volume per tissue volume (B), trabecular number (C), trabecular thickness (D) were assessed in the distal femur. (E) Atgl mRNA levels after ad-Cre mediated gene deletion in vitro. (F) Relative levels of [^{14}C]-Oleate oxidation to $^{14}CO_2$. (G) qPCR analysis of genes associated with osteoblastic differentiation. (H) Staining for alkaline phosphatase activity (AP) and calcium deposition by alizarin red (ARS) on day 14 of in vitro differentiation in cultures of control and ΔCpt2 osteoblasts. Quantification of relative ARS levels after extraction are shown. For in vivo studies, $n \geq 4$ mice per genotype. All data are shown as mean ± SEM

Supplementary Figure 3. Osteoblast-specific Cpt2 mutants (ΔCpt2) have normal glucose tolerance and insulin sensitivity. (A-B) Glucose tolerance testing of 12 week old male (A) and female (B) control and ΔCpt2 mice. (C-D) Insulin tolerance testing of 12 week old male (C) and female (D) control and ΔCpt2 mice (n = 5-14 mice). All data are shown as mean ± SEM.

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Supplementary Table I

Primers used in genotyping and qPCR analysis of mRNA levels

	Forward Sequence	Reverse Sequence
Genotyping Primers		
Oc-Cre	TCCTCAAAGATGCTCATTAG	GTAACTCACTCATGCAAAGT
	CAAATAGCCCTGGCAGAT	TGATACAAGGGACATCTTCC
Cpt2 flox	GCTGGCTTAGGAGATTCTTAACTTCC	AGCTCAGGTGGCAGAAATGATACC
Atgl flox	CGGTGAGGGTGGGGAACGGAGTC	CAGGGGGCCAGGCGGTCAGA
qPCR Primers		
18S	CTTAGAGGGACAAGTGGCG	ACGCTGAGCCAGTCAGTGTA
Cpt2	CCTGCTCGCTCAGGATAAACA	GTGTCTTCAGAAACCGCACTG
Runx2	CCAAATTTGCCTAACAGAATG	GAGGCTGTGGTTTCAAAGCA
Sp7 (Osx)	ATGGCGTCCTCTCTGCTTG	TGAAAGGTCAGCGTATGGCTT
Bglap2 (Osteocalcin)	GACAAAGCCTTCATGTCCAAG	AAAGCCGAGCTGCCAGAGTTT
Col I	GCATGGCCAAGAAGACATCC	CCTCGGGTTTCCACGTCTC
Ldha	CAAAGACTACTGTGTAACTGCGA	TGGACTGTACTTGACAATGTTGG
Ldhb	TGCGTCCGTTGCAGATGAT	TTTCGGAGTCTGGAGGAACAA
Slc2a1	GCCTGACCTTCGGATATGAGC	TGCCATAGCAGTCAATGAGGA
Atgl	GGATGGCGGCATTTCAGACA	CAAAGGGTTGGGTTGGTTCAG

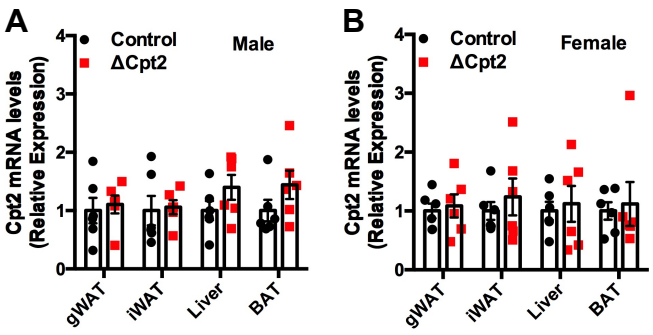
Supplementary Table II

Cellular Acylcarnitine Analysis

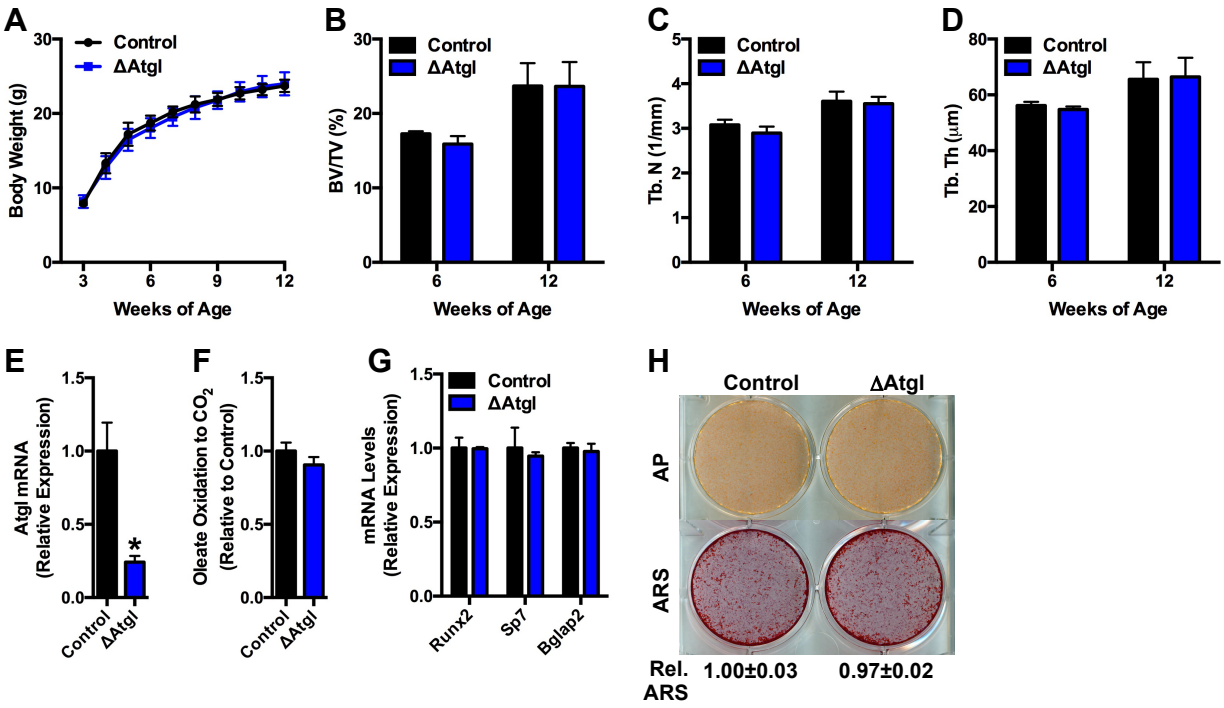
Acylcarnitines (pmol/mg protein)‡	Osteoblast Culture	
	Control	ΔCpt2
C0	92.28 ± 22.15	496.51 ± 49.54*
C2	272.44 ± 92.85	602.69 ± 33.95*
C3	2.53 ± 0.40	2.30 ± 0.37
C3-DC	3.38 ± 0.43	2.27 ± 0.35
C4	2.07 ± 0.31	4.59 ± 0.84*
C4-OH	2.44 ± 0.53	1.67 ± 0.18
C4-DC	0.57 ± 0.07	0.56 ± 0.10
C5:1	0.44 ± 0.09	0.31 ± 0.08
C5	6.37 ± 2.66	10.38 ± 1.56
C5-OH	0.95 ± 0.28	2.14 ± 0.35*
C5-DC/C10-OH	1.49 ± 0.11	0.64 ± 0.13*
C6	0.78 ± 0.20	0.72 ± 0.14
C8:1	0.67 ± 0.38	0.48 ± 0.10
C8	0.33 ± 0.11	0.25 ± 0.09
C10:1	0.21 ± 0.06	0.29 ± 0.07
C10	0.18 ± 0.07	0.35 ± 0.04
C12:1	0.49 ± 0.20	0.84 ± 0.10
C12	0.80 ± 0.14	2.08 ± 0.31*
C12:1-OH	0.28 ± 0.09	0.69 ± 0.13
C12-OH	1.10 ± 0.26	0.46 ± 0.07*
C14:2	0.27 ± 0.10	0.47 ± 0.05
C14:1	0.98 ± 0.22	5.98 ± 1.60*
C14	2.00 ± 0.48	28.50 ± 5.79*
C14:1-OH	0.84 ± 0.06	3.85 ± 0.46*
C14-OH	1.94 ± 0.37	0.98 ± 0.19
C16:1	2.17 ± 0.43	35.47 ± 6.04*
C16	12.48 ± 3.50	183.90 ± 14.11*
C16:1-OH	2.71 ± 0.68	11.53 ± 0.82*
C16-OH	3.95 ± 1.51	1.76 ± 0.24
C18:2	1.28 ± 0.30	7.53 ± 0.96
C18:1	10.59 ± 3.36	193.74 ± 16.75*
C18	14.44 ± 5.61	132.69 ± 9.20*
C18:2-OH	0.85 ± 0.42	1.82 ± 0.14
C18:1-OH	4.35 ± 1.94	1.84 ± 0.17
C18-OH	3.86 ± 1.83	1.33 ± 0.09

‡Values are shown as mean ± S.E.

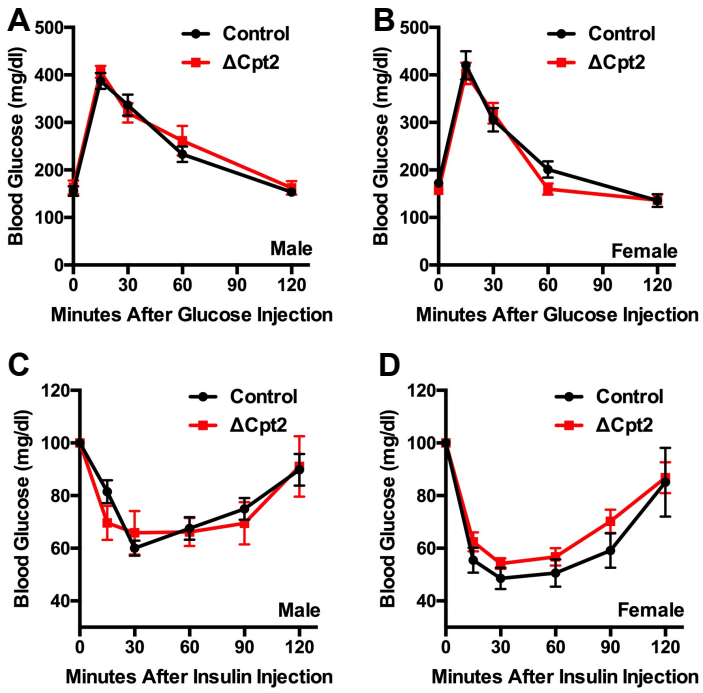
* $p < 0.05$



Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.