Supplemental Experimental Procedures

Recruitment of human subjects

Women with AN were recruited through on-line advertisements and referrals from local eating disorder providers and normal-weight controls were recruited through on-line advertisements. Subjects with AN met DSM-IV weight and psychiatric criteria. Normal-weight control subjects did not have a past or present history of an eating disorder and had a normal BMI, a history of regular menstrual cycles and were receiving no medications known to affect bone mass. Exclusion criteria included abnormal thyroid function tests, chronic diseases known to affect bone mineral density (other than AN) and diabetes mellitus. Subjects were examined by a physician and blood was drawn for laboratory studies during a visit at the Massachusetts General Hospital Clinical Translational Science Center. Height was measured as the average of three readings on a single stadiometer and subjects were weighed on an electronic scale while wearing a hospital gown. BMI was calculated using the formula [weight (kg)/height (meter)^2] and percent ideal body weight (%IBW) was calculated based on 1983 Metropolitan Life Height and Weight Tables (Editorial Submission, 1983). Data from a subset of subjects were previously published (Bredella et al., 2009; Fazeli et al., 2012; Fazeli et al., 2010).

Cancer therapy in human subjects

Women with ovarian and endometrial cancer receiving radiation and/or chemotherapy were recruited for a pilot study. The primary regimen for ovarian cancer patients was carboplatin with caclitaxel for at least 6 cycles. The majority of endometrial cancer patients were treated using external beam pelvic radiation therapy (EBRT) with a median dose of 45-50 cGy (in 25-28 fractions) and a 5-18 Gy additional radiation dose at the vaginal surface using high dose rate (HDR) brachytherapy radiation treatment.

Measurement of serum adiponectin in human subjects

For AN and HC subjects, levels of total, HMW, and HMW plus MMW adiponectin were measured using an ELISA (Alpco Diagnostics, Salem, NH) with a detection limit of 0.019 ng/mL, inter-assay coefficient of variation (CV) of 7.7% and an intra-assay CV of 6.6%. LMW adiponectin was calculated by subtracting HMW + MMW from total adiponectin; MMW adiponectin was then calculated by subtracting HMW from HMW + MMW adiponectin.

For subjects undergoing cancer therapy, total serum adiponectin was measured using an ELISA (Human Adiponectin Platinum ELISA, eBiosciences, San Diego, USA; Catalog no. BMS2032) with a detection limit of 0.01 ng/mL and an overall inter-assay CV of 3.1%.

Radiologic Imaging in human subjects

For AN and HC subjects, ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) of the L4 vertebral body, the proximal femoral epiphysis, metaphysis and mid-diaphysis was used to determine lipid content of the bone marrow using a 3.0T MR imaging system (Siemens Trio, Siemens Medical Systems, Erlangen, Germany) by methods previously described (Bredella et al., 2009). Subcutaneous adipose tissue (SAT), visceral adipose

tissue (VAT) and total adipose tissue (TAT) of the abdomen were determined using a single axial MR imaging slice through the abdomen at the level of L4, and SAT of the thigh was determined using a single slice through the mid-thigh (Siemens Trio, 3T, Siemens Medical Systems, Erlangen, Germany). Subjects underwent dual energy x-ray absorptiometry (DXA) to measure BMD of the PA lumbar spine (L1-L4), lateral spine (L2-L4), total hip, total body and % body fat using a Hologic Discovery A densitometer (Hologic Inc., Waltham, MA). Coefficients of variation of DXA have been reported as <1% for bone (Barthe et al., 1997). MAT mass was calculated as follows: *1)*, Total BM mass was calculated as 5% of ideal body weight (Hindorf et al., 2010); *2)*, For HC subjects MAT mass was calculated as 70% of total BM mass (Hindorf et al., 2010); *3)*, Based on measurements of L4 MAT, metaphysis MAT and diaphysis MAT (Table 1), AN subjects have 34% more MAT than HC subjects. Therefore, for AN subjects, MAT mass was calculated as 94% of total BM mass. MAT as percentage of total adipose mass was then calculated for each subject.

Subjects undergoing cancer therapy were imaged on a 3 T Siemens TRIO scanner (TIM Trio, Siemens, Erlangen, Germany) covering L1-L5 and Femoral Neck at baseline (after surgery but before radiation or chemotherapy), 6 months, and 12 months after treatment. Treatment changes were assessed by measuring marrow sFF [fat/(fat+water)] in the L5 vertebra, based on the detailed water-fat MRI method reported recently (Bolan et al., 2013). Body fat percentage was measured by DXA using a Lunar DPX-IQ (Lunar Radiation, Inc., Madison Wisconsin, USA) before and 12 months after treatment.

Rabbit bone analysis

To visualize the spatial distribution of MAT and red marrow *in situ*, tibiae and femurs were bisected using a Dremel rotary tool (Robert Bosch Tool Corporation, Addison, IL); a constant drip of sterile USP-grade water was used during cutting to prevent overheating.

Immunoblot analysis

To isolate total protein, tissues were first pulverized in liquid nitrogen using a pestle and mortar. Pulverized tissues were then mixed with lysis buffer (1% SDS, 12.7 mM EDTA, 60 mM Tris-HCl; pH 6.8) heated to 95 ºC, followed by homogenization by sequential passaging through 21-gauge and 26-gauge needles. Lysates were then centrifuged at 20,000 xg for 15 min at 4 ºC, lipid layers were discarded and supernatants transferred to fresh tubes and stored at -80 ºC. Estimation of protein concentration, SDS-PAGE and immunoblotting of tissue lysates was performed as described previously (Cawthorn et al., 2012). To detect total adiponectin in sera or conditioned media, samples were reduced and denatured by mixing with 4X SDS loading buffer, incubating at 95 ºC for 5 min, and cooling on ice for 1 min before separating by SDS-PAGE, as described previously (Du et al., 2013). To detect different multimeric complexes of adiponectin, sera or media were mixed with non-reducing running buffer (10 mM Tris-HCL pH 6.8, 0.6% SDS, 2% glycerol, 0.01% bromophenol blue) before separating by SDS-PAGE. The following antibodies were used: rabbit polyclonal anti-adiponectin (Sigma-Aldrich, A6354) for adiponectin in mouse lysates and

sera; mouse monoclonal anti-adiponectin (Thermo Scientific, MA1-054) for adiponectin in rabbit lysates; mouse monoclonal anti-adiponectin (R&D, Systems MAB10652) for adiponectin in human lysates or conditioned media; rabbit polyclonal anti-ERK1/2 (Cell Signaling, 9102); rat monoclonal anti-FABP4 (R&D Systems, MAB1443); rabbit polyclonal anti-HSL (Cell Signaling, 4107); rabbit polyclonal anti-Lamin A/C (Santa Cruz Biotechnology, sc-20681); mouse monoclonal anti-Perilipin A (Vala Sciences, 4854); rabbit polyclonal anti-PPARγ (Santa Cruz Biotechnology, sc-7273); rat monoclonal anti-α-tubulin (Thermo Scientific, MA1-80017); rabbit monoclonal anti-AMPKα (Cell Signaling, 2603); rabbit monoclonal anti-phospho-AMPKα Thr172 (Cell Signaling, 2535); rabbit monoclonal anti-LKB1 (Cell Signaling, 3047); rabbit monoclonal anti-phospho-LKB1 Ser428 (Cell Signaling, 3482); rabbit monoclonal anti-CaMKIIα (Cell Signaling, 3362); rabbit monoclonal anti-phospho-CaMKIIα Thr286 (Cell Signaling, 3361).

Real-time qPCR

Total RNA isolation, reverse transcription, primer design and quantitative PCR (qPCR) were performed as described previously (Cawthorn et al., 2012; Du et al., 2013). The following mouse primers have been described elsewhere: *Tbp, Adipoq, Fabp4,* and *Pparg* (Du et al., 2013); *Pgc1a* and *Tfam* (Mori et al., 2012); *Acadm* (Primer Bank ID 6680618a1); *Hnf4a* (Primer Bank ID 46575915c1; *Fabp5* (Primer Bank ID 6754450a1). All other primers are described in the following table.

Species	Transcript	Primer sequence (5'-3')
O. Cuniculus	*Adipoq*	(F) GCCACAATGGGACCCCAGGC (R) AGGACCAACAAGACCTGCATCTCC
	Cebpa	(F) TGGGCGGCATCTGCGAACAC (R) AAGGCGGCCGGGTCGATGTA
	Fabp4	(F) AAGTGGGAGTGGGCTTCGCC (R) GGGTTTGGCCATGCCAGCCA
	Lep	(F) GTCCAGGATGACACCAAGACC (R) CAGGGATGAAGTCCAAACCGA
	Pparg	(F) CGTGGATCTCTCGGTCATGG (R) TAATCCGCAACCATGGGGTC
	Ppia	(F) TCACCCACCTGACCATTCCT (R) GCTATTTGCAGACACGGAACC
	Tbp	(F) GACTCCTGGGATCCCCATCT (R) GTGTCAGTCCTGTGCCGTAA
M. musculus	*Fasn*	(F) GTCGTCTGCCTCCAGAGC (R) GTTGGCCCAGAACTCCTGTA
	Lep	(F) GACACCAAAACCCTCAT (R) CAGTGTCTGGTCCATCT
	Ppia	(F) CACCGTGTTCTTCGACATCA (R) CAGTGCTCAGAGCTCGAAAGT
	Ppara	(F) CCTGAACATCGAGTGTCGAATAT (R) TCTTCTTCTGAATCTTGCAGCT

Sequences of primers for qPCR.

Tissue culture

For analysis of adiponectin secretion from MAT and WAT of rabbits, tissues were dissected into 20-30 mg explants, with equal explant masses used for tissues from individual rabbits. Explants were then transferred to 24-well plates where they were washed with and then cultured in warm, sterile KRH buffer. Samples of media were collected after 1 h of culture, and again after 4 h of culture, and the adiponectin content of conditioned media determined by immunoblotting. Minimal explant breakdown was apparent after culture. Immunoblots in Figures 1F and S1C are from media after 4 h in culture.

For analysis of adiponectin secretion from MAT and scWAT of humans, tissues were dissected into 30-50 mg explants, with equal explant masses used for tissues from the same patient. Explants were then transferred to 48-well plates where they were washed with and then cultured in warm, sterile Dulbecco's modified Eagles' medium (catalog no. 11965; Gibco, Grand Island, NY) containing 100 units/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate, for 5-6 h in 5% CO_2. This duration of culture is consistent with that used in a previous study (Kovacova et al., 2009). Samples of media were collected after 45 min of culture, and again after 5-6 h of culture, and the adiponectin content of conditioned media determined by immunoblotting. Minimal explant breakdown was apparent after culture. Immunoblots in Figure 1H are from media after 5 h in culture; similar results were obtained for conditioned media after 45 min of culture.

Analysis of bone morphology by µCT

Mouse bones were embedded in 1% agarose and placed in a tube of 19 mm diameter. Bones were then scanned using a microCT system (µCT100 Scanco Medical, Bassersdorf, Switzerland) with the following settings: voxel size 12 µm, medium resolution, 70 kVp, 114 µA, 0.5 mm AL filter, and integration time 500 ms. Density measurements were calibrated to the manufacturer's hydroxyapatite phantom. Analysis was performed using the manufacturer's evaluation software. Mouse cortical bone was analyzed with a threshold of 280, as follows: 1, the growth plates and tibia/fibula junction were identified and the distance in slices between the two calculated; 2, 70% of this distance was calculated and added to the growth plate landmark; 3, contour at this slice; 4, contour 30 slices up from this initial slice; 4, iterate between these two contours using an outer value of 0 and an inner value of 280, using the stop button to stop. Mouse trabeculae were analyzed using a threshold of 180, as follows: 1, identify the growth plate and go down five slices; 2, draw an internal outline every 10 slices for 50 slices; 3, back-calculate using an outer value of 272 and an inner value of 0. The total volume of mouse bones was determined by contouring around the entire bone between the growth plate and tibula/fibula junction, and then calculating the bone volume and total volume using a threshold of 220.

Osmium tetroxide staining

BM adipocytes were labeled with osmium tetroxide as follows: *first*, bones were decalcified for 14 days in 14% EDTA, pH7.4; *second*, wash bones for 3 x 10 min in Sorensen's Phosphate buffer, pH 7.4, or in PBS; *third*, stain bones in 1% osmium

tetroxide for 24 h; *fourth*, wash bones for 3 x 4-6 h in Sorensen's Phosphate buffer, pH 7.4; *fifth*, re-scan bones with μCT, as described above; *sixth*, identify the tibia/fibula junction; *seventh*, determine the number of slices between the tibia/fibula junction and the growth plate in the same bone prior to decalcification, and then subtract this number to identify the growth plate on the osmium scan; *eighth*, use a threshold of 400 to quantify the marrow fat (exclude any fat in attached tissues) between the growth plate and tibia/fibula junction on the osmium-stained bone. Decalcification, osmium staining and wash steps were done at room temperature. We have recently described this method elsewhere (Scheller et al., 2014).

Statistical Analysis

Statistical significance was assessed as described under 'Experimental Procedures' in the main text. Error bars in figures represent SEM. For all comparisons, a *P*-value of < 0.05 was considered statistically significant.

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