Quantification of Human and Rodent Brown Adipose Tissue Function Using ^{99m}Tc-methoxyisobutylisonitrile SPECT/CT and ^{18}F-FDG PET/CT

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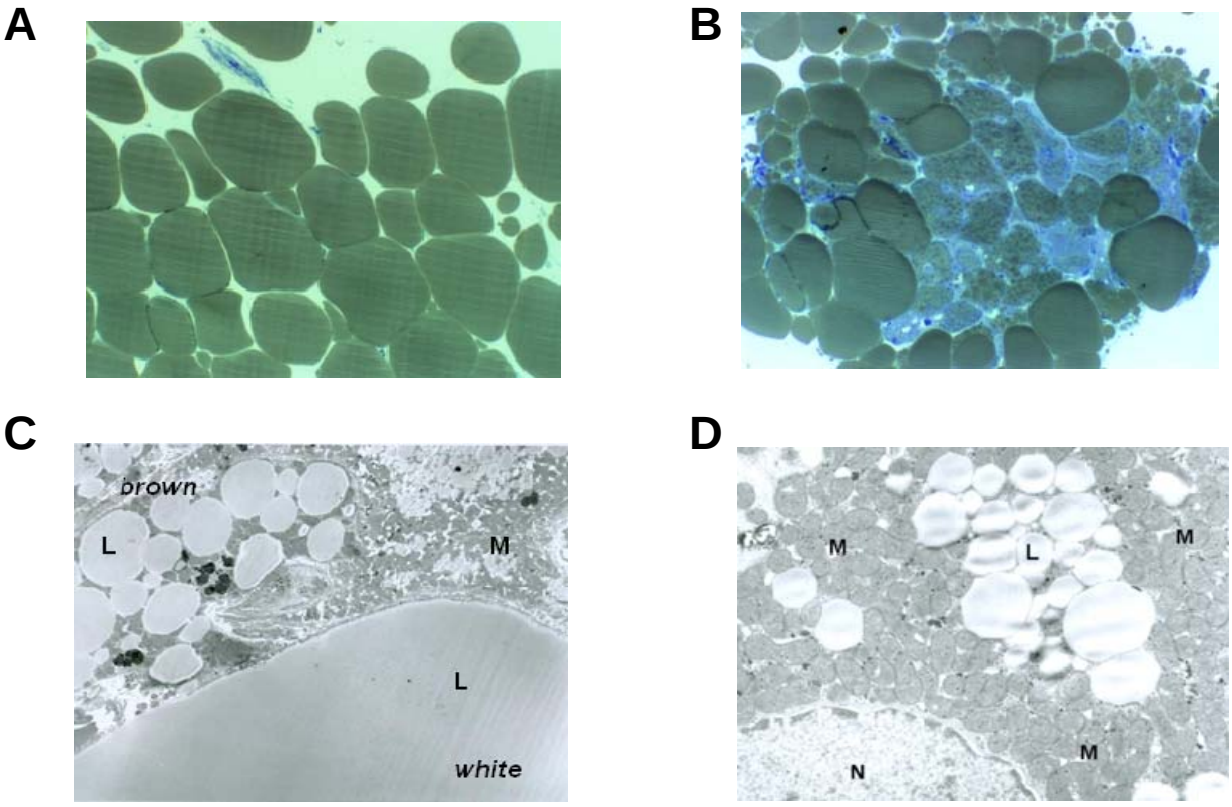
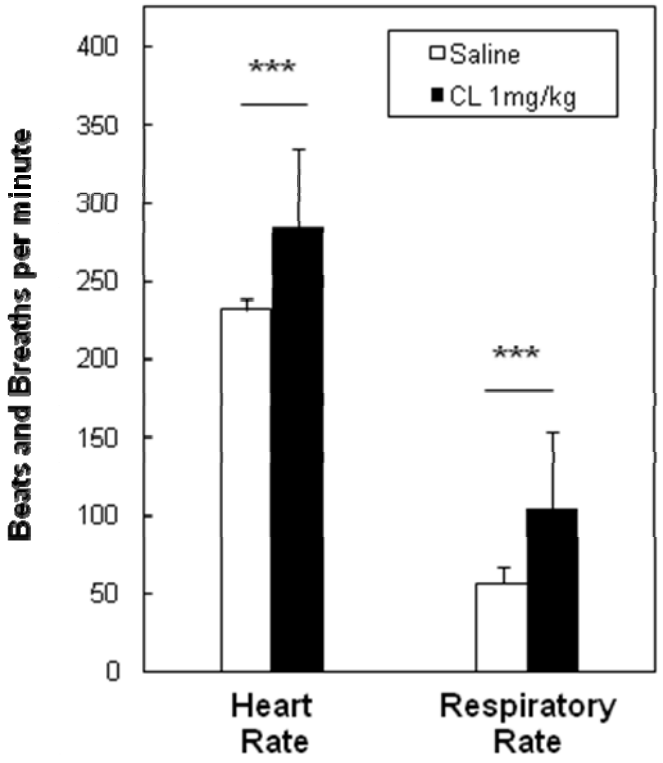
Supplementary Figures 1-2

Histological and Ultrastructural Appearance of Human White and Brown Adipose Tissue



Supplementary Figure 1. 57 year-old woman with clinical hyperparathyroidism. Osmium tetroxide staining of the biopsied tissue shows individual (A) white and (B) brown adipocytes. Electron micrographs showing (C) a brown (*upper*) and white (*lower*) adipocyte; (D) higher magnification of mitochondria and lipid droplets. Labels are the following: lipid droplets (L), mitochondria (M), and nucleus (N).



Supplementary Figure 2. Male 129SVE mice, 8-12 weeks-old, were treated with the β3-adrenergic receptor agonist CL-316,243 (CL) (1mg/kg) (n=15) or saline control (n=16). Effects on heart rate and respiratory rate post CL injection were measured over 30 minutes. ***, $P < 0.001$.

Supplementary Table 1. Primer sequences used in quantitative RT-PCR for lineage marker analysis.

Gene		Sequences
UCP-1	Forward	5'-ACCGCAGGGAAAGAAACAGC-3'
	Reverse	5'-TCAGATTGGGAGTAGTCCCT-3'
DIO2	Forward	5'-AGTGCAGAAGGAGGTGACAACAGT-3'
	Reverse	5'-AAAGTCAAGAAGGTGGCATGTGGC-3'
CideA	Forward	5'-CCTTTCCGGGTCTCCAAC-3'
	Reverse	5'-CATCTTCCTCCAGCACCAGA-3'
PGC1α	Forward	GGAATCCTGAGCGGTACGATG
	Reverse	CTGGCAGGTGTATGTCCCATT
Leptin	Forward	5'-TCTATGTCCAAGCTGTGC-3'
	Reverse	5'-TTGGAGGAGACTGACTGC-3'

Supplementary Methods

Human Imaging

All patients were injected with 20 mCi (740 MBq) of ^{99m}Tc-MIBI and planar dual-phase imaging was performed with standard parameters after 20 minutes and 2 hours using a Philips Medical Systems (Andover, MA) Precedence SPECT/CT. The hybrid SPECT/CT consists of a dual-head gamma camera and a diagnostic capability 6-slice CT scanner. The gamma camera consists of 2 detectors with a field of view of 40 × 54 cm with 3/8-in. Ultra-high resolution and low sensitivity collimators were used with a 180° detector angle. Matrix size was 128 × 128 over a 360° arc with a 2.8° step and step-and-shoot acquisition: 20 seconds duration per frame, 64 stops, zoom factor of 1, and noncircular orbits. Images were reconstructed from raw data to transaxial slices by back projection with a Hann filter. Sagittal and coronal slices were then generated. Total acquisition time was approximately 25 minutes. The CT system consists of a 6 slice system. Acquisition parameters were: collimation 6 × 1.5 mm, pitch 0.85, gantry rotation 750 milliseconds, field of view 600 mm, tube current 75 mA, and tube voltage 120 kV. Images were reconstructed with 5-mm slice width with 5-mm increments using filtered back projection (matrix: 512 × 512). No IV contrast was administered for the acquisition. Images were used for attenuation correction and anatomic localization. Static views were done 20 minutes after tracer injection, then SPECT/CT imaging after the 20-minute static views, and finally 2-hour planar views.

Animal Imaging

Imaging was performed on a NanoSPECT/CT (Bioscan, Washington, DC) and a NanoPET/CT (Mediso Medical Imaging Systems, Budapest, Hungary), each equipped with an 8 W X-ray source running at 55 kVp (145 mA) and a 48 μm pitch CMOSCCD X-ray detector. Continuous helical micro-CT scanning was employed with the following parameters: 0.5 s exposure, 240 angles, 1.3 magnification, 37 mm pitch (1 field-of-view), and a 512 x 256 pixel frame size (192 μm pixels). Images were reconstructed as 170 x 170 pixel transverse matrices with varying axial length and slice thickness of 0.4 mm (isotropic voxel size 0.4 mm) using filtered-back projection (SheppLogan filtering). Helical micro-SPECT was performed using a four-headed gamma camera outfitted with multi-pinhole collimators 1.0mm diameter pinholes for a single mouse scan. Images were acquired over 360 angles in 48 projections of 50 s each using a 256 x 256 frame size (1.0 mm pixels). The micro-SPECT images were reconstructed as 86 x 86 pixel transverse matrices with varying axial length and slice thickness of 0.8 mm (isotropic voxel size 0.8 mm). Static PET was acquired for 30 min using with a 1:3 coincidence mode, 5 ns coincidence time window, normal coincidence rate mode and fine timestamp list mode type.

FOV was 94.7mm, with 50% axial overlap. Reconstruction was performed using Nucline software (Mediso Medical Imaging Systems, Budapest, Hungary) in 2D mode, matrix size 149x150x150mm for a final voxel size of 0.2925mm. OSEM reconstruction algorithm was used with a SSRB 2DLOR rebinning method; further reconstruction parameters include a 400-600keV energy window, 1:1 coincidence mode, and with a ring difference of 16. ^{18}F-FDG and ^{99m}Tc-sestamibi uptake was assessed using InVivoScope software (Bioscan, Washington, DC).