Supplemental Information to:

Browning of Subcutaneous White Adipose Tissue in Humans

EXPERIMENTAL PROCEEDURES

Human and mouse sample collection

sWAT samples were collected from the torso or limbs of healthy and severely burned children and adults during their scheduled surgeries while they were under general anesthesia. For burn patients, burn wounds were excised down to the healthy adipose tissue to provide a viable wound bed for skin grafting. sWAT samples were taken from the exposed healthy sWAT. sWAT was sampled from the torso or limbs depending on the location of the skin graft procedure. For healthy children and adults, sWAT samples were taken during their elective surgery from the same incision. Subcutaneous fat was harvested from the abdomen (n=6), neck (n=1), lower back (n=2) and buttocks (n=1) of healthy children and the subcutaneous abdominal WAT of healthy adults (n=9). Human supraclavicular WAT was harvested from healthy adult humans by a positron emission tomography–computed tomography (PET-CT) guided percutaneous needle biopsy following acute (4-6hr) cold exposure and administration of the radio-active isotope ^{18}F-flurodeoxyglucose. Subcutaneous WAT was sampled from the abdomen.

The intrascapular BAT of male balb-c mice (8-12 weeks) were harvested from animals immediately after euthanasia by exposure to a rising CO_2 concentration. Animals were housed in a 12-12 light dark cycle at ~ 25°C.

Histology and immunohistochemistry analysis

The sWAT samples were dehydrated, embedded in paraffin and cut into 5 µm sections. The slices were stained with hematoxylin and eosin for histology. Immunohistochemistry was performed according to a standard protocol. Upper airway mucus deposition in lung tissue of burn trauma victims. Shock 2008;29:356-61). Briefly, the paraffin was removed from the WAT sections with xylene and rehydrated with a series of alcohol solutions. For heat-induced epitope retrieval, the sections were immersed in a preheated citrate buffer solution at 95°C for 20 min. The sections were treated with hydrogen peroxide solution to mask the activity of endogenous peroxidase. After blocking with goat serum (rabbit IgG kit, Vector Laboratory, Burlingame,CA, USA) for 45 min, the sections were incubated overnight with the primary antibody (rabbit anti-UCP1 antibody, Sigma u6382, Sigma-Aldrich, St. Louis, MO, USA) at a dilution of 1:1600 at 4°C. The following day, the sections were incubated with a secondary antibody (Rabbit IgG kit, Vector Laboratory, Burlingame, CA, USA), avidin-biotin - complex (ABC) solution, and then a diaminobenzidine solution (DAB peroxidase substrate kit, Vector Laboratory, Burlingame, CA, USA) at room temperature. We used phosphate-buffered saline with Tween 20 (0.2%) to wash sections between each step. The sections were counterstained with hematoxylin. We created negative control slides by substituting buffer for the primary antibody.

Mitochondrial leak respiration

sWAT samples (100-200 mg) for mitochondrial respiration analysis were placed in preservation medium (10 mM EGTA buffer containing 5.8 mM ATP, 6.6 mM $MgCl_2$,

20 mM taurine, 20 mM imidazole, 0.5 mM dithiothreitol and 50 mM MES) and immediately transferred to the laboratory where they were blotted on filter paper and weighed. From each sample, approximately 50 mg of WAT was transferred to Oxygraph respirometer chambers (Oroboros Instruments GmbH, Innsbruck, Austria) and suspended in 2 ml of respiration medium (0.5 mM EGTA buffer containing 20 mM HEPES, 10 mM potassium phosphate, 60 mM potassium lactobionate, 3 mM $MgCl_2$,20mMtaurine, 110 mM sucrose and 1 mg/ml bovine serum albumin) (for human and mouse BAT measurements, 2-5 mg of tissue was used). After the chambers were sealed, 2 µM digitonin was injected into each one to permeabilize the adipocyte cell membranes. All measurements were made at 37°C and at O_2 concentration greater than 100 nmol/ml.

Briefly, after the oxygen flux had stabilized within the respirometry chambers, oligomycin insensitive uncoupled mitochondrial respiration was determined. To do this, pyruvate (5 mM), octanoyl-carnitine (1.5 mM), malate (2 mM), glutamate (10 mM), followed by ADP (5mM) and then succinate (10mM) were added to the Oxygraph chamber to achieve coupled state 3 respiration. Then 5µM oligomycin was titrated into the Oxygraph chamber to achieve leak state 4_O respiration. In the results, we present state 4_O respiration and the coupling control ratio for oligomycin, which was determined by dividing state 4_O respiration by state 3 respiration.

Activity of citrate synthase

The activity of citrate synthase was measured as a proxy of mitochondrial density. Tissue homogenates (25 mg/ml) were prepared in 175 mM potassium chloride containing 2 mM EDTA and 1% Triton. Following homogenization (15-30 sec in a glass tissue grinder), the samples were frozen and thawed twice to ensure destruction of the cells and membranes. After subsequent centrifugation (3500 $\times$ g for 2 min at 4°C), the supernatants were recovered and stored at -80°C until analysis. The maximum activity rates of citrate synthase were determined in extracts diluted 1:20 in a 0.1 M phosphate buffer (pH 7.1) containing 30 mM acetyl-CoA and 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Following the addition of 10 mM oxaloacetate, light absorbance was monitored with a BioTek Eon™ spectrophotometer (Winooski, VT, USA) in a kinetic mode for 10 min at 412 nm. The free coenzyme A produced from the condensation of acetyl-CoA and oxaloacetate within the tricarboxylic acid cycle is bound to DTNB. The resulting change in light absorbance, detected at 412 nm, was used to determine the activity of citrate synthase (nmol/g/sec).

Figure Legends

Supplemental Figure 1. Whole body REE following burn trauma (related to Figure 4).

Absolute REE strongly correlated with age **(A)** and body mass **(B)** in our patient cohort, but negatively correlated with age when normalized to body mass **(C)**. Indeed, when absolute REE was correlated with days post burn, no relationship between REE and days post burn was observed **(D)**. However, when REE was normalized to body mass, we found a significant correlation with REE and days post burn **(E)**.

93 **Supplemental Figure 1.**

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