Life Sciences 89 (2011) 78–85

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Effects of burn injury, cold stress and cutaneous wound injury on the morphology and energy metabolism of murine brown adipose tissue (BAT) in vivo

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ARTICLE INFO

Article history:
Received 2 November 2010
Accepted 9 April 2011

Keywords:
Brown fat
PET
Metabolism

ABSTRACT

Aims: Cold stress has been shown to produce dramatic increases in 2-fluoro-2-deoxy-D-Glucose (^{18}FDG) accumulation by brown adipose tissue (BAT) in rodents. However, neither the effects of other types of stress on ^{18}FDG accumulation nor the effects of stressors on the accumulation of tracers of other aspects of energy metabolism have been evaluated. In this report we studied the effects of cold stress, burn injury and cutaneous wounds on murine BAT at the macroscopic, microscopic and metabolic level.
Main methods: Glucose metabolism was studied with ^{18}FDG, fatty acid accumulation was evaluated with trans-9(RS)-^{18}F-fluoro-3,4(RS,RS)-methyleneheptadecanoic acid (FCPHA) and tricarboxcylic acid cycle (TCA) activity was evaluated with ^{3}H acetate.
Key findings: All three stressors produced dramatic changes in BAT at the macroscopic and microscopic level. Macroscopically, BAT from the stressed animals appeared to be a much darker brown in color. Microscopically BAT of stressed animals demonstrated significantly fewer lipid droplets and an overall decrease in lipid content. Accumulation of ^{18}FDG by BAT was significantly ($p<0.01$) increased by all 3 treatments (Cold: ~16 fold, burn ~7 Fold and cutaneous wound ~14 fold) whereas uptake of FDG by white fat was unchanged. This effect was also demonstrated non invasively by μPET imaging. Although less prominent than with ^{18}FDG, BAT uptake of FCPHA and acetate were also significantly increased by all three treatments. These findings suggest that in addition to cold stress, burn injury and cutaneous wounds produce BAT activation in mice.
Significance: This study demonstrates brown fat activated by several stressors leads to increased uptake of various substrates.

Introduction

Brown adipose tissue (BAT) and white adipose tissue (WAT) are both found in mammals. The primary function of WAT is lipid storage whereas BAT is intimately involved in energy metabolism. BAT is especially abundant in newborns and in hibernating mammals (Cannon and Nedergaard, 2004) where its primary function is to generate body heat by "non-shivering thermogenesis". The mechanism of this process is believed to be related to uncoupling of substrate utilization and ATP production in the mitochondria, with resulting dissipation of metabolic energy as heat (Sell et al., 2004).

Until recently, the interpretations of ^{18}FDG-PET studies were confounded by the presence of focal areas of increased tracer accumulation in the supraclavicular region, intercostal region, peri-adrenal region, axilla and around the great vessels which were falsely ascribed to nodal disease. With the introduction of PET/CT, these findings were confirmed to represent focal areas of BAT and not nodal disease. These findings were shown to be most prominent in lean females during the cold months (Van Marken Lichtenbelt et al., 2009; Cypess et al., 2009; Virtanen et al., 2009; Saito et al., 2009; Au-Yong et al., 2009).

Cold stress has also been shown to activate ^{18}FDG uptake in rodents (Tatsumi et al., 2004), however, neither the effects of other types of stress on ^{18}FDG accumulation nor the effects of stressors on the accumulation of tracers of other aspects of energy metabolism

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doi:10.1016/j.lfs.2011.04.014

have been evaluated. BAT has also been considered to be important in insulin resistance, which correlates with impaired insulin signaling in peripheral tissues (Valverde and Benito, 2005).

In the present report, we studied the effects of the cold stress, burn injury and cutaneous wounds to mice on BAT at the macroscopic level, microscopic level and the metabolic level [^{18}FDG accumulation (glycolysis), FCPHA accumulation (fatty acid utilization) and ^{3}H-acetate accumulation (TCA cycle activity)].

Overall, our findings indicated that cold stress, burn injury and cutaneous wounds have significant effects on BAT at the structural and functional levels.

Materials and methods

Materials

^{18}F labeled FDG was prepared by routine methods (Hamacher et al., 1986). Trans-9(RS)-^{18}F-fluoro-3,4(RS,RS)-methyleneheptadecanoic acid (FCPHA) was prepared using a procedure developed in our laboratory (Shoup et al., 2005). $^{3}H^{-}$ Acetate, SOLVABLE™ and Ultima Gold™ were purchased from Perkin Elmer Inc. (Waltham MA).

Animal preparation

Male CD-1 mice weighing ~28 g (Charles River, Wilmington MA) were used in these studies. After acclimatizing to the MGH animal facility for at least five days, groups of six animals were treated as follows:

Burn injury

Full-thickness, non-lethal thermal injury (30% total body surface area [TBSA]) was produced, as described previously (Zhang et al., 2005). Briefly, the mice were anesthetized with ether and their backs were shaved with animal hair clippers. Under ether anesthesia, the mice were placed in molds exposing 30% TBSA followed by emersion of the exposed area in a water bath at 90 °C for 9 s. The animals were immediately resuscitated with saline (15 ml/kg) by intraperitoneal injection. Sham animals were treated similarly with the exception that the water bath at 90 °C was replaced with room temperature water. After the procedure, the animals were caged individually and fasted 24 h at room temperature with free access to water prior to radiopharmaceutical administration.

Cold stress

To produce cold stress, the mice were placed in a cold room at 4 °C for 24 h with overnight fasting with free access to water. The mice were housed three to a cage in wire mesh bottom cages and the radiopharmaceuticals were administered on the following morning.

Cutaneous wounds

For this procedure, the mice were anesthetized with ether, their backs were shaved with animal hair clippers and a 1 cm^2 section of skin was removed to the level of the fascia to produce a full thickness wound. After the procedure, the mice were housed individually in wire mesh bottom cages and fasted 24 h with free access to water at room temperature prior to radiopharmaceutical administration.

Animal care was provided in accordance with the procedures outlined by the National Institutes [National Institutes of Health. Guide for Care and Use of Laboratory Animals (Department of Health and Human Services Publication 85-23). Bethesda, MD, National Institutes of Health, 1996]. The study was approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

Biodistribution studies

The fasted animals were injected (without anesthesia) via tail vein with ^{18}FDG (50.0 μCi), FCPHA (50.0 μCi) or ^{3}H-acetate (5.0 μC). One hour after administration of ^{18}FDG and FCPHA or 30 min after administration of ^{3}H-acetate the animals were sacrificed, selected tissues were excised, weighed and biodistribution was determined by direct measurement of tissue radioactivity using a Wizard Gamma counter. Tissues containing ^{3}H-acetate were added to 1 ml of SOLVABLE™ in a glass vile which was capped and heated at 60 °C with shaking. The samples were then cooled and decolorized with 0.3 ml of 30% hydrogen peroxide. 10 ml of Ultima Gold™ was added to each sampler and radioactivity was measured with a Beckman LS6000 lC liquid scintillation counter. Radioactivity in aliquots of the injected doses were counted with the tissue samples to correct for radioactive decay. All results were expressed as % injected dose per gram of tissue (%ID/g, mean ± sem).

μPET imaging

Additional groups of control and stressed animals were studied by ^{18}FDG microPET (μPET) imaging, performed with a P4 μPET camera (Concord Microsystems Inc. Knoxville, TN). One hour after intravenous injection of ^{18}FDG (~500.0 μCi) via tail vein without anesthesia, the mice were anesthetized, positioned and stabilized in the gantry of the PET camera and a 10 min image was acquired in list mode. The primary imaging characteristics of the P4 camera are in-plane and axial resolutions of 1.75 mm FWHM, 63 contiguous slices of 1.21 mm separation and a sensitivity of ~650. In all animals, the region from the head to the base of the tail was included in the 7.9 cm field of view of the camera. The PET images were reconstructed using an iterative algorithm (Ordered Subset Expectation Maximization, OSEM). Data for attenuation correction was measured with a rotating point source containing ^{68}Ge. All projection data were corrected for non-uniformity of detector response, dead time, random coincidences, and scattered radiation. The PET camera was cross-calibrated to a well scintillation counter by comparing the camera response from a uniform distribution of an ^{18}F solution in a 5.0 cm cylindrical phantom with the response of a well counter to an aliquot of the same solution.

Histology

The BAT lobules and contiguous or nearby normal WAT tissues were excised from the posterior cervical-upper thoracic region and immersed in 10% formalin. After 24 h of fixation, the excised fat was examined, comparative changes were noted and lobe sizes were measured. Tissues were then block sectioned, inserted into cassettes, processed to paraffin blocks, microtome sectioned to 6 μm and stained with H&E (hematoxylin and eosin) for microscopic examination.

The H&E slides were evaluated microscopically for histological changes in BAT and adjacent WAT tissues. Lipid content (dissolved and removed by xylene during paraffin block processing) was represented by distinctive clear intra-cytoplasmic vacuoles. The lipid content was estimated as a percentage of “clear areas” relative to remaining areas of stained cellular components (nucleus and cytoplasm) and supporting connective tissue. A calibrated ocular grid was used on random fields and percentages were calculated as statistical averages. This method was utilized in lieu of fat stained frozen tissue sections which are cumbersome to evaluate and fraught with a host of staining artifacts. Additionally, this method permitted equal or greater accuracy.