Characterization of Brown Adipose Tissue in Mice with IDEAL Fat-Water MRI

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INTRODUCTION – Brown adipose tissue (BAT) remains a topic of interest in obesity research, due to its role in thermogenesis, body weight regulation, and lipid metabolism [1-4]. Whereas adipocytes in white adipose tissue (WAT) contain a *single* large intracellular lipid droplet with limited cytoplasm and vascular supply, BAT adipocytes are characterized by *multiple* lipid droplets, an abundance of mitochondria, a rich cytoplasm, and a well-developed set of vascular and neural networks. Due to these differences in cellular and tissue structures, it has been shown that the fat fraction **(FF)** index from IDEAL fat-water MRI can be used to non-invasively differentiate BAT from lipid-rich WAT, based on the *lower* FF of BAT [5]. In this work, we first demonstrate with IDEAL in mice the ability to locate various BAT depots. We then demonstrate that IDEAL FF can be used to characterize BAT in mice that have been housed at cooler (19°C) and warmer (25.5°C) temperatures. We hypothesize that a reduced ambient temperature will *increase* BAT activity in the cold-19°C-stimulated mice. Consequently, these thermogenically-activated BAT depots will result in *lower* FF than in the warm-25.5°C-housed animals.

METHODS – All MRI exams were performed on a GE 3 Tesla human scanner using a wrist T/R coil. Multi-fat-peak 3D IDEAL [6] was used with the following parameters: 0.5 mm isotropic voxel, BW=±125 kHz, flip angle=7°, TR=10ms, ΔTE=0.8ms, and first TE=1.5-1.8ms. ***Identification of BAT.*** Twenty-two mice (2 to 19 weeks old) were imaged to locate and corroborate various BAT depots previously identified in the literature from necropsy [4]. For anatomical reference, cryosection was performed on a few specimens. Specimens were skinned and fixed in 10% buffered formalin overnight at 4°C, followed by immersion in a 30% sucrose solution (0.1M phosphate buffer, pH ~7.4). Specimens were then imbedded in Tissue-Tek® Optimum Cutting Temperature solution, frozen in liquid nitrogen (-80°C), and sectioned with a Leica CM1900 cryostat. ***Temperature Experiment.*** Eleven additional C57Bl/6 age-matched mice were singly housed for three consecutive weeks at either 19°C (n=5; 4F/1M) or 25.5°C (n=6; 4F/2M). The mice were provided *ad libitum* access to food and monitored weekly for food intake and body weight. At 6-weeks of age, they were euthanized and imaged.

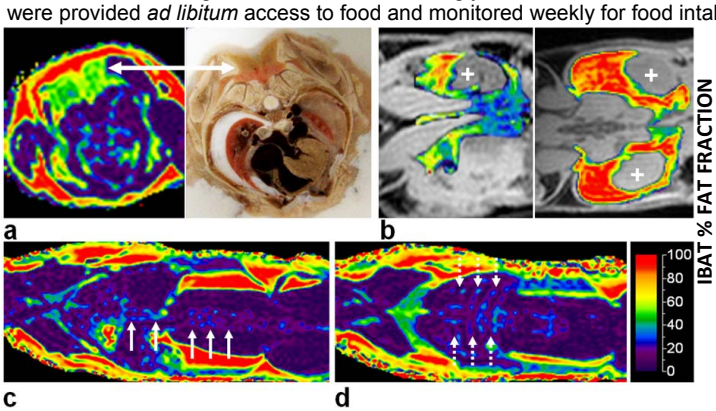

FIG. 1: **(a)** Axial fat fraction map with interscapular BAT (arrow) and a reference cryosection slice. **(b)** Peri-renal BAT surrounding the kidneys (+), with coronal fat fraction maps overlaid on top of water images in a 4 (left) and a 12-week old (right) mouse. **(c)** Indications of peri-aortic and para-spinal BAT (arrows), and **(d)** intercostal BAT (dashed arrows). Note color bar to represent FF: 0% fat– black; 100% fat – red.

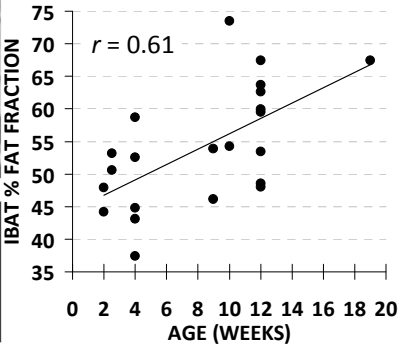

FIG. 2: Plot of average interscapular BAT (IBAT) fat fraction vs. age, showing a moderate trend of leaner (lower fat fraction) BAT in young mice and fattier BAT in adult mice. This is likely due to their different thermogenic demands.

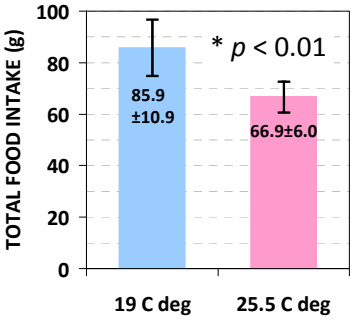

FIG. 3: Total average food intake (in grams) of the two mice groups over the three-week temperature housing period. There was a significantly greater (29%) amount of food intake by the 19°C animals.

RESULTS – ***Identification of BAT.*** FIG. 1 illustrates several BAT depots. FF maps reconstructed from IDEAL are in color (0% fat–black, 100% fat–red). Lipid-rich WAT is denoted in red. Panel (a) highlights the prominent and largest interscapular **(IBAT)** depot. The bi-lobed shape of the depot along the axial view agrees well with a reference cryosection slice. Panel (b) shows peri-renal BAT depots surrounding the kidneys. FF maps are superimposed on top of IDEAL water images. In the adult mouse (right), the depot is very WAT-like (high FF); in the juvenile mouse (left), the FF is much lower. Panels (c, d) are coronal FF maps highlighting the peri-aortic, para-spinal, and intercostals depots, along the animal. FIG. 2 plots the average IBAT FF vs. mice age. The trend suggests lower BAT FF in young mice than in adults. This is potentially indicative of higher thermogenic requirement in juveniles due to their greater surface-volume ratio and body heat loss. Their BAT is thus more actively engaged in thermogenesis, which leads to increased lipid metabolism (∴ lower FF).

Temperature Experiment. Housing mice at 19°C vs. 25.5°C resulted in an *increase* (29%, $p < 0.01$) in food intake over the three-week stimulation period (FIG. 3), *despite similar* body weight gain, final body weight, and body composition ($p > 0.05$). FIG. 4 illustrates the average IBAT FF between the two groups, showing a 12% lower FF in the 19°C (range: 35.2-48.6%) vs. the 25.5°C group (range: 48.4-60.9%). Note the WAT layer in the 25.5°C animal surrounding the BAT; this is nearly absent in the 19°C animal. There were no significant group differences between the IBAT depot volumes (19°C: 53.7±23.4 mm^3 vs. 25.5°C: 51.7±17.4 mm^3).

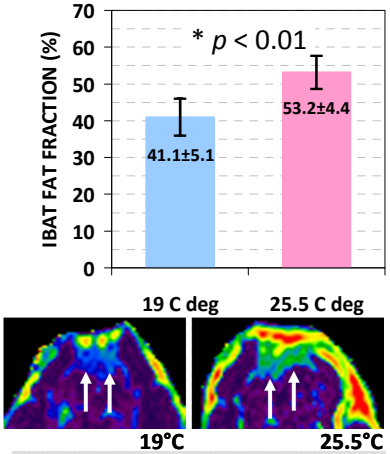

FIG. 4: IBAT FF for the two groups, showing a significant 12% lower FF in the 19°C mice. Note evident variations in the example axial FF maps of IBAT (arrows, same color bar as FIG.1).

CONCLUSION – We have shown that IDEAL can non-invasively measure BAT depots in mice. We have also shown that mice subjected to cold-temperature stimulation have *lower* BAT FF than those housed at a warmer temperature. The greater thermal demand on the 19°C animals was accompanied by increases in food intake and BAT activity, without significant weight or fat gain differences, in comparison to the 25.5°C mice. These findings are in support of known cellular and thermogenic adaptions of BAT in mice. We thus conclude that IDEAL FF imaging will be useful in detecting measurable BAT differences, and in monitoring tissue activity in longitudinal studies of BAT stimulation. Future work will extend to characterization of human BAT using IDEAL.

REFERENCES – [1] Cinti. *Nutr Metab and Cardio Dis* 16:567-574, 2006. **[2]** Nedergaard, et. al. *Am J Physiol Endocrin Metab* 293:E444–E452, 2007. **[3]** Himms-Hagen, et al. *Can Med Assoc J* 121:1361-1364, 1979. **[4]** Cannon B, et al. *Physiol Rev* 84:277-359, 2004. **[5]** Hu H, et al. ISMRM 2009, 210. **[6]** Yu H, et al. *MRM* 60:1122-1134, 2008. ***The first two authors contributed equally to this work.***