Chemical-Shift MRI Measurements of Variations in Murine Brown Adipose Tissue Fat Content Due to Housing Temperature

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INTRODUCTION: Brown adipose tissue (BAT) is a topic of strong interest in obesity research due to its role in non-shivering thermogenesis, energy balance, and metabolism. In rodent models, decreased BAT activity has been associated with an increased risk of diet-induced obesity (e.g. positive energy balance, resulting in weight gain and accumulation of white fat) and diabetes. Nearly all animal studies of BAT involve sacrifice of the animals, extraction of the BAT depots, and characterization of the tissue by histology. Thus, the ability to non-invasively assess BAT *in vivo* remains an unmet need. Previous works have demonstrated the feasibility of MRI to identify and characterize BAT in mice **[1, 2]**. In this study, we utilize chemical-shift MRI and its fat-signal fraction metric to measure *in vivo* the differential fat content of BAT in mice housed at cold, room, and warm temperatures. We hypothesize that a *reduction* in ambient housing temperature leads to an *increase* in BAT thermogenic activity, and thus a *decrease* in BAT fat content.

METHODS: Forty-eight 8-week old male C57BL/6J mice were singly housed in environmental chambers (Powers Scientific, Pipersville, PA) set to either 16° (cold ***C***), 23° (room ***R***), or 30° (warm ***W***) Celsius (n=16/group). Mice were provided *ad libitum* access to standard rodent chow (Teklad Global 16% Protein Rodent Diet, Harlan, Madision, WI) and autoclaved water, with 12:12 hour light-dark cycles. Total body weight and food intake for each animal were measured weekly throughout the duration of the four-week study. Total body lean and fat mass were also determined by the quantitative magnetic resonance (QMR) method (Echo Medical Systems, Houston, TX) at baseline, week 2, and week 4, for each animal. At the end of week 4, MRI was performed on an animal 9.4T system (BioSpec, Bruker BioSpin) using a single channel surface coil to measure interscapular BAT fat-signal fraction in each animal. Anesthesia (2% isoflurane) was given throughout each scan and respiratory gating was utilized. A three-echo chemical-shift water-fat sequence based on the IDEAL technique **[3]** was utilized, involving a 2D GRE sequence with TR=295ms, TEs=2.8/3.8/4.8ms, flip angle=10°, FOV=3cm, 128x128 acquisition matrix, and 1mm slices. Eight (no gap) slices were used to cover the dorsal interscapular BAT depot and the average scan time per animal with respiratory gating was ~8 minutes. At 9.4T, the resonance frequency difference between water and methylene fat protons is ~1.36kHz and an echo spacing of 1ms translates to ~480° phase shifts (4x the optimal water-fat phase shift of $2\pi/3$ as described in IDEAL **[4]**).

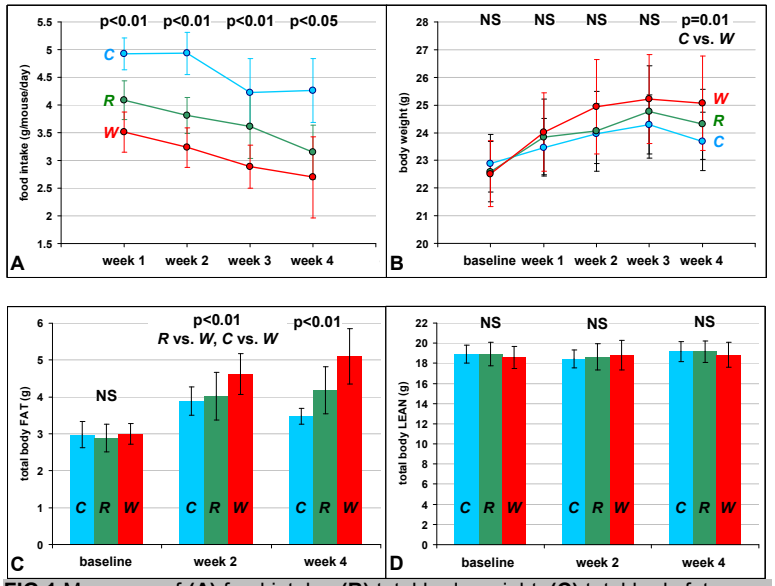

FIG.1 Measures of **(A)** food intake, **(B)** total body weight, **(C)** total body fat mass, and total body lean mass across 16°C-housed ***C***, 23°C-housed ***R***, and 30°C-housed ***W*** animals.

RESULTS: Measures of food intake (g), body weight (g), lean and fat mass (g), and fat-signal fraction (%) are reported as group means. Fig. 1A illustrates an evident inverse relationship between housing temperature and food intake. Mice in the 16°C group (***C***) consumed ~33%, ~41%, ~37%, and ~45% more food than those in the 30°C (***W***) group during weeks 1-4, respectively. However, as shown in Fig. 1B, despite the differential food intake, total body weights between any of the 3 groups (***C***, ***R***, and ***W***) were not significantly different at baseline or during weeks 1-3. During week 4, only the body weights between the ***C*** and ***W*** groups were significantly different. Note furthermore in Fig. 1B that the ***W*** group gained body weight the quickest from baseline to week 4, with the most noticeable increase during the first two weeks. Fig. 1C and 1D summarize body composition results from QMR, demonstrating that the body weight gains observed in Fig 1B was primarily attributed to fat mass. Fig.1C shows that while there were no significant difference in total body fat mass at baseline between the three groups, significant differences manifested progressively by weeks 2 and 4. Conversely, Fig. 1D illustrates that there were minimal changes and differences in total body lean mass between the three groups throughout the experiment. Fig. 2 plots the interscapular BAT fat-signal fraction from chemical-shift MRI, illustrating a 10-20% difference between the three groups and a strong positive association with ambient housing temperature. Representative axial fat-signal fraction color maps of interscapular BAT from different mice are shown for each group.

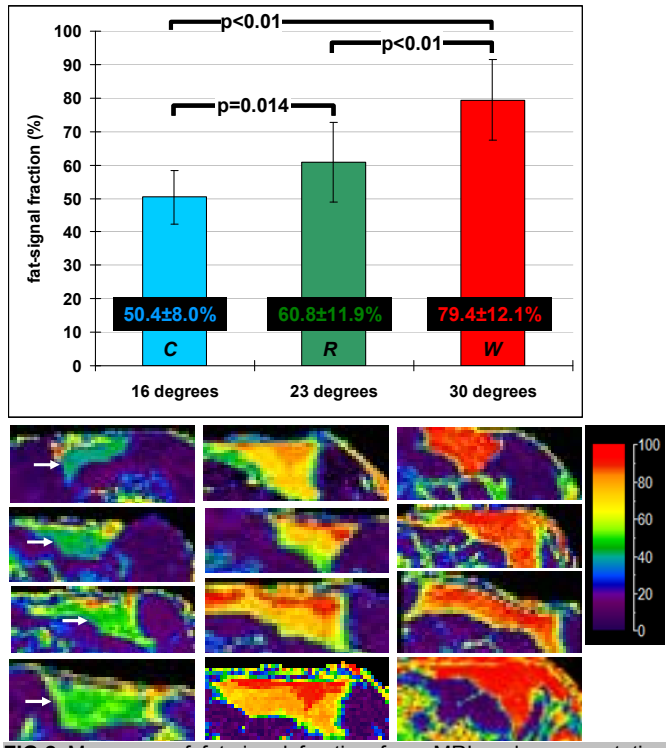

FIG.2 Measures of fat-signal fraction from MRI and representative axial images from each animal group. Arrows point to BAT depot.

CONCLUSION: Results from this study systematically demonstrate the differential thermal demand of animals in each housing temperature group and the utility of chemical-shift MRI to non-invasively measure and reflect BAT activity *in vivo*. Coincident with the highest BAT fat content (lowest activity), animals in the 30°C-housed ***W*** group exhibited the greatest gains in body weight (predominantly fat mass) despite the lowest food intake. Conversely, animals in the 16°C-housed ***C*** group exhibited the lowest BAT fat content (highest activity), smallest body weight gains, but yet the greatest food intake.

REFs: [1] Hu, JMRI 2010;31:1195-1202. **[2]** Branca, MRM 2011;65:313-319. **[3]** Reeder, MRM 2004;51:35-45. **[4]** Pineda, MRM 2005;54:625-635.