Longer T2 relaxation time is a marker of hypothalamic gliosis in mice with diet-induced obesity

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Lee D, Thaler JP, Berkseth KE, Melhorn SJ, Schwartz MW, Schur EA. Longer T2 relaxation time is a marker of hypothalamic gliosis in mice with diet-induced obesity. Am J Physiol Endocrinol Metab 304: E1245–E1250, 2013. First published April 2, 2013; doi:10.1152/ajpendo.00020.2013.—A hallmark of brain injury from infection, vascular, neurodegenerative, and other disorders is the development of gliosis, which can be detected by magnetic resonance imaging (MRI). In rodent models of diet-induced obesity (DIO), high-fat diet (HFD) consumption rapidly induces inflammation and gliosis in energy-regulating regions of the mediobasal hypothalamus (MBH), and recently we reported MRI findings suggestive of MBH gliosis in obese humans. Thus, noninvasive imaging may obviate the need to assess MBH gliosis using histopathological end points, an obvious limitation to human studies. To investigate whether quantitative MRI is a valid tool with which to measure MBH gliosis, we performed analyses, including measurement of T2 relaxation time from high-field MR brain imaging of mice fed HFD and chow-fed controls. Mean bilateral T2 relaxation time was prolonged significantly in the MBH, but not in the thalamus or cortex, of HFD-fed mice compared with chow-fed controls. Histological analysis confirmed evidence of increased astrocytosis and microglial accumulation in the MBH of HFD-fed mice compared with controls, and T2 relaxation times in the right MBH correlated positively with mean intensity of glial fibrillary acidic protein staining (a marker of astrocytes) in HFD-fed animals. Our findings indicate that T2 relaxation time obtained from high-field MRI is a useful noninvasive measurement of HFD-induced gliosis in the mouse hypothalamus with potential for translation to human studies.

MATERIALS AND METHODS

Animals. Animals were maintained in a temperature- and humidity-controlled room on a 12:12-h light-dark cycle and were housed and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols, animal handling, and treatment were approved by the Institutional Animal Care and Use Committee of the University of Washington. Group-housed male C57BL/6 mice (8 –10 wk) were assigned to HFD (60% of kilocalories from fat; LabDiet 5001). Mice were maintained in a temperature- and humidity-controlled room on a 12:12-h light-dark cycle and were housed and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols, animal handling, and treatment were approved by the Institutional Animal Care and Use Committee of the University of Washington. Group-housed male C57BL/6 mice (8–10 wk) were assigned to HFD (60% of kilocalories from fat, D12492; Research Diets) or standard laboratory chow (12% of kilocalories from fat; LabDiet 5001). Mice were given ad libitum access to their assigned diet (HFD, n = 8; control, n = 8) for 21 wk, and body weight was recorded weekly. During weeks 20 and 21, mice underwent body composition analysis, MR imaging, and a terminal perfusion, each separated by ≥2 days.

Body composition analysis. In vivo analysis of body lean mass, fat mass, and water content was performed in conscious, immobilized mice by quantitative magnetic resonance (EchoMRI 3-in-1 Animal Tissue Composition Analyzer; Echo MRI, Houston, TX) (20).

MRI procedures. Mice underwent isoflurane anesthesia in an induction chamber. Once in an appropriate plane of anesthesia, eye lubricant was applied, the mice were placed on a bite bar, and their heads were placed into a radiofrequency coil and secured to a cradle created specifically for the MRI system. The coil was then inserted vertically into a scanner heated to maintain thermoneutrality (32°C). The coil is equipped with an adjustable anesthetic flow and vacuum system to maintain sedation throughout the experiment. Total scan time was 1–1.5 h, during which respiration was monitored through a respiration sensor under the abdomen (SA Instruments, Stony Brook, NY) and anesthesia titrated to ensure appropriate sedation. Following the imaging paradigm (described below), mice were removed from the

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Acquired using a single-slice, multiecho sequence. A T2 map was created by calculating T2 values using an exponential fit function, where $D_{ij}$ and $D'_{ij}$ are diffusion tensor components for the laboratory frame of reference and the tissue frame of reference, respectively. The MBH slice of interest was determined at the time of acquisition for T2 maps based on a high-resolution multislice, multi-echo sequence (Table 1 and the description above). DTI slice of interest to the slice selected for the T2 map-scanning sequence.

Regions of interest (ROIs) were placed in the bilateral MBH, thalamus, and cortex. One of the eight mice from the chow-fed group was excluded from the DTI analyses (fractional anisotropy and tensor trace) due to an image artifact present in ROIs.

**Immunohistochemistry.** After imaging was completed, mice were perfused with 4% paraformaldehyde-PBS, and 14-μm-thick frozen sections in the coronal plane through the mouse hypothalamus were obtained on a cryostat and processed for glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) immunoreactivity using standard immunohistochemical procedures. Sections blocked in 5% normal goat serum (Jackson Immunoresearch Laboratories) were incubated overnight at 4°C with mouse Cy3-conjugated anti-GFAP (1:10,000; Sigma-Aldrich) and rabbit anti-Iba1 (1:1,000; Wako Pure Chemicals). Coinmunofluorescence was performed by adding Alexa Fluor 488-labeled anti-rabbit secondary antibody (1:500; Invitrogen). GFAP and Iba1 antibodies have been widely validated in the literature as markers for astrocytes and microglia, respectively (19).

Images were captured on an Eclipse E600 upright microscope equipped with a color digital camera (Nikon). Quantification and ROI placement within the ARC of the MBH were performed in a blinded fashion on 20 immunofluorescence images. Both sides of bilateral structures were examined on two adjacent sections per animal, and replicate values from each animal were averaged individually before group means were determined ($n = 8/group$). To quantify astrocytosis, mean GFAP immunostaining intensity was calculated for individual ROIs using Image J (http://rsbweb.nih.gov/ij/). For microglial counts, Iba1 immunostaining allowed identification of discrete cells that were counted manually within the prespecified ROIs. Total microglial count was determined by summing right and left sides and then averaging this value from the two sections examined. For microglial density, thresholding was performed in Image J, followed by densitometric quantification.

**Statistical analysis.** Group means ± SE were determined for body composition variables. Right and left values were averaged together for each animal to provide a total measurement for each region and are

### Table 1. High-resolution MRI protocol for quantitative assessment of gliosis in mouse MBH

<table>
<thead>
<tr>
<th>Method</th>
<th>Sequence Type</th>
<th>TR/TE, ms</th>
<th>FOV, mm</th>
<th>Acquisition/Reconstruction Resolutions</th>
<th>Acquisition Time</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scout imaging</td>
<td>GRE</td>
<td>30/1.3</td>
<td>30</td>
<td>~10 s</td>
<td></td>
<td>3 orthogonal images used for animal positioning</td>
</tr>
<tr>
<td>Image planning</td>
<td>Multislice RARE</td>
<td>668/4.5, 15 slices</td>
<td>20</td>
<td>~1 min</td>
<td></td>
<td>Slice position and orientation selected</td>
</tr>
<tr>
<td>T2w</td>
<td>Multislice RARE</td>
<td>4,000/10, 15 slices</td>
<td>17</td>
<td>66 × 66 μm</td>
<td>8 min</td>
<td>To cover MBH and determine a slice of interest</td>
</tr>
<tr>
<td>T2 map</td>
<td>Single-slice, multiecho</td>
<td>4,000/6-75, 12 echoes, 1 slice</td>
<td>17</td>
<td>66 × 133/66 × 66 μm</td>
<td>12 min</td>
<td>Fast T2 maps to observe T2 changes between high fat diet- and chow-fed mice</td>
</tr>
<tr>
<td>Diffusion</td>
<td>DTI-EPI</td>
<td>4,000/18 ms, 4 shot EPI, 30 diffusion directions, multislice</td>
<td>19</td>
<td>150 × 150 μm</td>
<td>9 min</td>
<td>FA and diffusion tensor trace maps to monitor changes in brain microstructural integrity</td>
</tr>
</tbody>
</table>

TR, recycle delay; TE, echo time; FOV, field of view; GRE, gradient echo; δ, flip angle; SE, spin echo; RARE, rapid acquisition with refocused echoes; T2w, T2 weighted; DTI-EPI, diffusion tensor imaging-echo planar imaging; 2D, two-dimensional; FA, fractional anisotropy; MBH, mediobasal hypothalamus.
reported as such unless otherwise indicated. Unpaired t-tests were used to test for group differences in body weight, body composition, and histopathological measurements. Two-way analysis of variance was used for analyses that examined the effect of diet (HFD vs. chow) and region (thalamus, cortex, MBH) on MR-derived outcomes and the interaction of those factors or that assessed laterality within a specific ROI. Pearson’s correlation coefficients were determined, and linear regression models were used to determine significant associations among MR, body composition, and histological measures. Results were considered significant at \( P < 0.05 \). All statistical analyses were performed with GraphPad Prism (La Jolla, CA) version 5.04.

RESULTS

Body composition. At the time of imaging (21 wk of diet), HFD-fed mice were slightly heavier than chow-fed mice (31.9 ± 0.75 vs. 30.0 ± 0.39 g; \( t_{14} = 2.21, P = 0.04 \)) but had a substantially higher percentage of fat mass (12.8 ± 1.9 vs. 7.76 ± 0.5%; \( t_{14} = 2.55, P = 0.02 \)) and lower percentage of lean body mass (82.8 ± 1.6 vs. 87.8 ± 0.9%; \( t_{14} = 2.73, P = 0.02 \)).

**T2 relaxation time.** ROIs and example images from the high-resolution 2D sequence, T2 parametric map, and DTI-EPI sequence are shown in Fig. 1. There was a significant effect of diet (\( F_{1,42} = 4.42, P = 0.04 \)), region (\( F_{2,42} = 8.09, P = 0.001 \)), and an interaction between diet and region for \( T_2 \) relaxation time (\( F_{2,42} = 3.84, P = 0.03 \)), indicating that the effect of diet to significantly increase \( T_2 \) relaxation times was dependent on the region (Fig. 2A). Region-by-region analyses confirmed that HFD-fed animals had modestly but significantly longer \( T_2 \) relaxation times in the MBH (30.5 vs. 28.9 ms, \( t_{14} = 2.93, P = 0.01 \)) but not in the thalamus (28.3 vs. 28.4, \( t_{14} = 0.33, P = 0.75 \)) or cortex (29.5 vs. 29.2, \( t_{14} = 0.49, P = 0.63 \)). The effect of HFD to lengthen \( T_2 \) relaxation time in the MBH persisted when the left and right MBH were included separately in the model (\( F_{1,28} = 12.6, P = 0.001 \)). Laterality (right vs. left) had no effect (\( F_{1,28} = 0.68, P = 0.42 \)), nor was there evidence for an interaction between side and diet (\( F_{1,28} = 0.56, P = 0.46 \)).

**Diffusion imaging.** Using diffusion tensor imaging, values were compared for fractional anisotropy and tensor trace assessments. For fractional anisotropy (Fig. 2B), there was a significant effect of region (\( F_{2,39} = 3.28, P < 0.05 \)) but no effect of diet (\( F_{1,39} = 0.33, P = 0.57 \)). For tensor trace

**Fig. 1.** Regions of interest and representative images of scan parameters. A: high-resolution 2-dimensional rapid acquisition with refocused echoes image for a slice selected to include the midregion of the arcuate nucleus. B: \( T_2 \) map generated from multiecho sequence. C and D: diffusion tensor imaging for fractional anisotropy (C) and tensor trace measurement (D). Regions indicate areas of analysis. R, right; L, left; MBH, mediobasal hypothalamus. Scale bar, 1 mm.

**Fig. 2.** Results of multiparametric imaging in high-fat diet (HFD)-fed mice and chow-fed controls. A: \( T_2 \) relaxation time is higher in HFD-fed animals in the MBH (\( t_{14} = 2.93; *P = 0.01 \)) but not in the control regions of the thalamus and cortex (diet × region interaction: \( F_{2,42} = 3.84, P = 0.03 \)). B: there were no diet effects for fractional anisotropy, but there was an effect of region (\( F_{2,39} = 3.28, P < 0.05 \)). C: there were no group differences in tensor trace measurements; \( n = 8 \) for each group except in fractional anisotropy and tensor trace analyses, where \( n = 7 \) for HFD due to an image artifact.
measurements (Fig. 2C), there was no effect of diet \( (F_{1,39} = 0.83, P = 0.37) \) or region \( (F_{2,39} = 0.08, P = 0.92) \).

**Histopathological correlation.** Animals fed a HFD had increased GFAP staining density in the MBH compared with chow-fed controls \( (t_{14} = 2.68, P = 0.02; \text{Fig. 3, A, B, and E}) \). Mean GFAP density from the MBH was negatively associated with percent lean mass \( (r = -0.54, P = 0.03) \) but was not as strongly associated with body weight or percent adipose mass \( (r = 0.38 \text{ and } P = 0.15, r = 0.37 \text{ and } P = 0.16, \text{respectively}) \). T2 relaxation time in the right MBH was positively associated with mean GFAP density in this same region, although this trend did not achieve significance \( (r = 0.48, P = 0.06; \text{data not shown}) \). However, when stratified by diet, T2 relaxation times in the right MBH were positively correlated with mean intensity in HFD-fed animals (Fig. 3F). No correlations were significant between intensity of GFAP staining and fractional anisotropy or tensor trace values (data not shown).

Total bilateral microglial number in the MBH was increased significantly in HFD- compared with chow-fed mice \( (t_{14} = 2.58, P = 0.02; \text{Fig. 3, C, D, and G}) \). Although not significant, microglial number tended to correlate with T2 relaxation time \( (r = 0.43, P = 0.09) \). No group difference in total density of microglial staining was present \( (3.22 \text{ vs. } 3.22, r_{14} = 3.24, P = 1.0), \) nor was Iba1 staining density significantly correlated with T2 relaxation time overall \( (r = 0.1, P = 0.7) \) or in stratified analyses considering HFD-fed mice only (data not shown).

**DISCUSSION**

Our findings add to growing evidence of MBH damage and gliosis induced by HFD feeding in mice and validate MR imaging as a tool for detecting and quantifying this effect in vivo. On high-field MR images, we found that T2 relaxation time was selectively prolonged in the MBH but not in the thalamus or cortex of HFD- vs. chow-fed mice. These data are consistent with gliosis (3, 4, 7, 13), and they extend findings of high MBH T2 signal in association with obesity in humans (19). Histologically, we found increased density of both astrocyte cell bodies and processes as well as increased microglial cell number in the ARC of HFD-fed mice. T2 relaxation time was significantly correlated with a marker of astrocytosis (GFAP density), but not with microglial density, among the HFD-fed mice, suggesting that the MRI technique we employed may be sensitive primarily to the astrocyte component of the gliosis response. These findings identify T2 relaxation time measured using high-field MRI as a promising quantitative radiological marker of gliosis in the MBH despite the minute size of this brain area. Because MRI is a safe and established tool for human brain imaging, these techniques justify studies that investigate the potential translational value of comparable measurements of gliosis in the MBH of obese humans.

The multiparametric MR approach we used allowed us to assess the utility of two other techniques based on diffusion tensor imaging that also have the potential to detect and quantify gliosis localized to mouse MBH (12). However, unlike the T2 relaxation signal, neither of the diffusion tensor imaging sequences that we employed detected the significant differences in MBH gliosis between groups. There are several possible reasons why diffusion tensor imaging measurements did not show any significant differences. The simplest explanation is that changes in brain microstructural integrity do not occur during HFD feeding in mice. Another possibility relates to the larger voxel size (150 vs. 66 \( \mu \)m for the T2 acquisitions) required for diffusion tensor imaging to reduce acquisition

![Fig. 3. Histopathological analyses. A–D: representative images of immunofluorescence analysis of MBH sections obtained from mice fed chow (A and C) or HFD for 21 wk (B and D). A and B: glial fibrillary acidic protein (GFAP) immunoreactivity (red) marks astrocyte cell bodies and processes. C and D: ionized calcium binding adaptor molecule 1 immunoreactivity (green) identifies microglial cells and their processes. E: mean arcuate nucleus GFAP staining intensity (determined by densitometry) was higher in HFD- compared with chow-fed mice \( (t_{14} = 2.68) \). F: T2 relaxation time in the right MBH was positively correlated with mean GFAP staining intensity from the right ARC in HFD-fed mice. G: bilateral ARC microglial number was higher in HFD- compared with chow-fed mice \( (t_{14} = 2.58) \). Representative regions of interest used for quantification of astrocyte and microglial cells within the ARC of the MBH are indicated by solid lines in A–D. Scale bar in A represents 50 \( \mu \)M. 3V, 3rd ventricle. *P = 0.02.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00020.2013)
time. This fact, combined with the use of only two diffusion gradients \((b\text{ values of } 0\text{ and }1,000 \text{ s/mm}^2)\), may have limited our ability to detect small changes in brain microstructure. Moreover, there were slight differences between ROI sizes for diffusion tensor imaging and T2 relaxation time that further complicate direct comparisons among the modalities. Therefore, it is possible that diffusion tensor imaging will yield informative data if modified approaches are applied. Additional MR approaches to consider in future studies include 1) T2 flair and inversion recovery sequences with white matter nulling to highlight signal changes and 2) assessment of magnetization transfer effects that may provide a measure of white matter integrity. Further optimization of these methods will not only enable the acquisition of information that is otherwise difficult to obtain, such as the ability to monitor dynamic changes in MBH gliosis over time in individual animals, but also have important translational implications for performing comparable studies in humans.

Previous studies (4, 7, 10) as well as our supplementary histopathological correlations suggest that longer T2 relaxation time reflects increased glial cell numbers (4, 7), reactive astrogliosis (10), and/or decreased neuronal populations (4) in the MBH. The relationship between T2 relaxation time and increased astrocyte density was particularly strong in the right MBH among HFD-fed animals, and trends were present among all animals. These findings suggest a positive correlation between MBH T2 relaxation times and astrocyte density. In contrast, there were no consistent relationships between microglial number or staining density and MBH T2 relaxation time despite the effect of the HFD to increase microglial cell number in the MBH. Although it is possible that astrocytosis rather than microgliosis is the primary gliosis component responsible for prolongation of the T2 relaxation time by HFD feeding in mice, other factors may also have affected the correlation between the T2 signal and histopathological end points. For one, our high-resolution MR sequences achieved 66 \(\mu\text{m}\) in-plane resolution in a 200-\(\mu\text{m}\)-thick slice, whereas the immunofluorescence sections had sub-\(\mu\text{m}\) resolution in a 14-\(\mu\text{m}\)-thick section. Thus, the fact that a much larger area of tissue contributed to the measurement of T2 relaxation time than to the histological end points undoubtedly contributed to variability in the statistical correlation between these measurements.

Several additional limitations and challenges remain to be addressed. In addition to uncertainty regarding the specific cell types involved in gliosis that increase T2 relaxation time, unmeasured histological changes in MBH tissue such as edema or enhanced vascularity could also affect the T2 signal (15, 24). Finally, the ARC has no distinctive appearance on MRI and is more difficult to target than to the histological end points undoubtedly contributed to variability in the statistical correlation between these measurements.

In summary, we report that T2 relaxation time as measured by high-field MRI is a useful, noninvasive tool for assessing MBH gliosis in mouse models of diet-induced obesity. This technique may be particularly useful for studies in which changes in MBH gliosis are measured serially over time to better define the time course and potential reversibility of hypothalamic damage in mouse models. MRI is also a safe and accessible tool that provides an exceptional opportunity to translate findings from rodent models to humans. Such studies are critical to establish whether hypothalamic inflammation, gliosis, and damage in humans, as in rodents (8, 19), is induced by obesity and/or dietary factors. Ultimately, this information may help to better understand the role of MBH gliosis in the pathogenesis of human obesity.

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DISCLOSURES

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS


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