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BRIEF ARTICLE

^{1}H-MRS Quantitation of Age-Dependent Taurine Changes in Mouse Brain

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Abstract

Purpose: A mouse model of Alzheimer's disease demonstrates reduced beta-amyloid levels in the whole brain, associated with a gain of hippocampal memory, after drinking taurine-enriched water; this suggests that a taurine supplement could be a promising treatment for cognitive deficit. The objective of this study is to establish a methodology for quantifying taurine in the whole brain, taking advantage of the rapid development of non-invasive imaging techniques such as magnetic resonance imaging and magnetic resonance spectroscopy (MRS).

Procedures: Single-voxel proton MRS was used to obtain quantifiable taurine peaks at 3.25 and 3.43 ppm. Quantitative MRS results were obtained in C57BL/6 mice of various age groups: 4, 11, 18, and 27 months old.

Results: Compared with the 4-month-old group, taurine levels dropped significantly only at 27 months of age ($p = 0.03$). However, a significant decrease of N-acetyl-aspartate (NAA) in the brain was observed at both 18 and 27 months ($p = 0.03$ and $p = 0.02$). In addition, MRS-measured taurine level is highly correlated with hippocampal volume ($r = 0.95$).

Conclusions: These results suggest that decreased taurine levels in the brain could be used as biomarkers for hippocampal changes and are fully translatable into putative cognitive loss in both animal models and human studies without the *ex vivo* approach.

Key Words: Taurine, Aging, MRI, Hippocampus, Magnetic resonance spectroscopy (MRS), Alzheimer's disease (AD)

Introduction

Approximately 44 million people worldwide currently face many complex challenges caused by dementia. Based on recent data, the global economic cost of dementia is estimated to be around $604 billion (USD) [1]. Despite this huge clinical need, there are currently no effective ways to slow down or reverse dementia. Interest in the possible therapeutic effect of taurine on dementia emerged since drinking taurine-containing water induces increased learning and memory in a mouse model of human Alzheimer's disease [2]. After drinking taurine-containing water at a dose level of 1000 mg/kg/day for 6 weeks, 7-month-old male APP/PS1 mice display reduced beta-amyloid levels in the whole brain and improved hippocampal memory by behavioral tests, thus suggesting that taurine supplement could be effective for neuroprotection in this model system [2].

Taurine is the second most abundant amino acid in the brain [2, 3]. In general, taurine levels in the brain decrease rapidly during early development and then decline modestly with age [4]. Taurine plays multiple roles, including thermoregulation, stabilization of protein folding, anti-inflammatory effects, antioxidation, osmoregulation, calcium homeostasis, CNS development, *etc.* [2–5]. In addition, taurine levels in the brain significantly increase under

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stressful conditions [6], suggesting that taurine may play a vital role in neuroprotection. A possible mechanism of taurine neuroprotection, seen in animal studies [2, 5], lies in its modulatory effects on both cytoplasmic and intra-mitochondrial calcium homeostasis [5, 7, 8]. Taurine also increases hippocampal neurogenesis in aging mice [9].

Until recently, taurine levels and distribution in the brain have been studied by *ex vivo* amino acid analysis, in extracted tissue samples [4]. Using a similar sample preparation approach, taurine can also be analyzed by LC-MS [10] or high-resolution ^{1}H-NMR [11]. These approaches are hardly feasible for clinical application for real-time changes in the brain. This problem has been overcome by the recent development of non-invasive imaging techniques such as magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), affording detection and quantification of changes in metabolite levels in the brain in both clinical and preclinical settings [12, 13]. For example, high-resolution MRS has been used to study adult and neonatal rat brain from birth to 28 days postnatal [14, 15]. Changes of brain metabolite concentrations measured by proton MRS during aging in Alzheimer's disease mice up to 18 month old were reported [16, 17].

We adapted this methodology, both MRI and proton MRS (^{1}H-MRS) assays, to evaluate age-dependent metabolic changes in C57BL/6 male mice at young, middle age, young-old, and old-old stages (4, 11, 18, and 27 months of age). To our knowledge, the current study is the first one to measure *in vivo* brain metabolites using advanced MRS methods in aging C57BL mice, covering the entire spectrum of 4 to 27 months of age.

Materials and Methods

Animals

All animal procedures used in this study were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC Protocol # 16520), in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Four age groups of C57BL/6 male mice were included in the study (4, 11, 18, and 27 months, $n = 6$ for each group). Mice were bred and maintained to the above age groups in our animal facility, with standardized 12 h light and dark cycles. Just before imaging, mice were anesthetized with 4 % isoflurane in O_2 and then maintained with 1.5 % isoflurane during MRI and MRS scans.

MRI and Quantification of Hippocampal Volume

MR imaging and spectroscopy data were acquired using a 9.4 T horizontal bore MRI system (Agilent Inc., Santa Clara, CA, USA). A 72-mm volume coil and a 2-channel mouse brain surface coil were used for RF transmission and detection (RAPID MR International, Columbus, OH, USA). T2-weighted anatomical images were obtained using a standard spin echo multi-slice (SEMS) imaging sequence, with the following parameters: TR/TE = 1600/23 msec, matrix size = 256 × 256, field of view (FOV) = 18 × 18 mm^2, and 31 slices with a slice thickness of 0.5 mm. After loading T2-weighted images into ImageJ (Version 1.47, NIH, Bethesda, MD, USA), the hippocampal region was manually segmented slice by slice to estimate the area of each slice. The total volume of the hippocampus was estimated by (total areas of hippocampus from all slices × slice thickness).

^{1}H-MRS and Quantification of Brain Metabolites

For localized ^{1}H-MRS data acquisition, a voxel was positioned at the center of the mouse brain, under the guidance of T2-weighted images described previously. The chosen voxel included most of the hippocampal region, as well as part of the thalamus (Fig. 1, top graph). Localization by adiabatic selective refocusing (LASER) sequence was used for MRS data acquisition with the following parameters: TR/TE = 1700/40 msec, voxel size = 2.2 × 3.3 × 3.0 mm^3, spectrum width = 4006 Hz, complex points = 2048, and number of averages = 256 (with 2 initial dummy scans). The LASER sequence provides minimal chemical shift displacement errors (voxel shift), as well as uniform excitation profile, using high bandwidth adiabatic pulses [18]. Variable power and optimized relaxation delay (VAPOR) was applied as a water suppression scheme [15]. All MRS data were loaded into spectrum analysis software package jMRUI (version 5.0, Université Claude Bernard, Lyon, France) for spectroscopy analysis and peak quantification. We used creatine (Cr) as the reference peak for all metabolite quantifications, based on the widely accepted assumption that the creatine level in the brain is relatively stable [19].

Statistical Analysis

All results were expressed as mean values ± standard deviation (SD). The paired Student's t test was used for comparison of data derived from two groups. Values with $p < 0.05$ were considered statistically significant.

Results

Brain Taurine Changes Measured by ^{1}H-MRS

An example of MRS data from the 27-month age group is shown in Fig. 1, representing the quality consistently achieved in this study. Major metabolites are readily seen with sufficient signal-to-noise ratio (SNR) to be reliably quantified. The single-voxel MRS was positioned in the central region of the brain, covering most of the

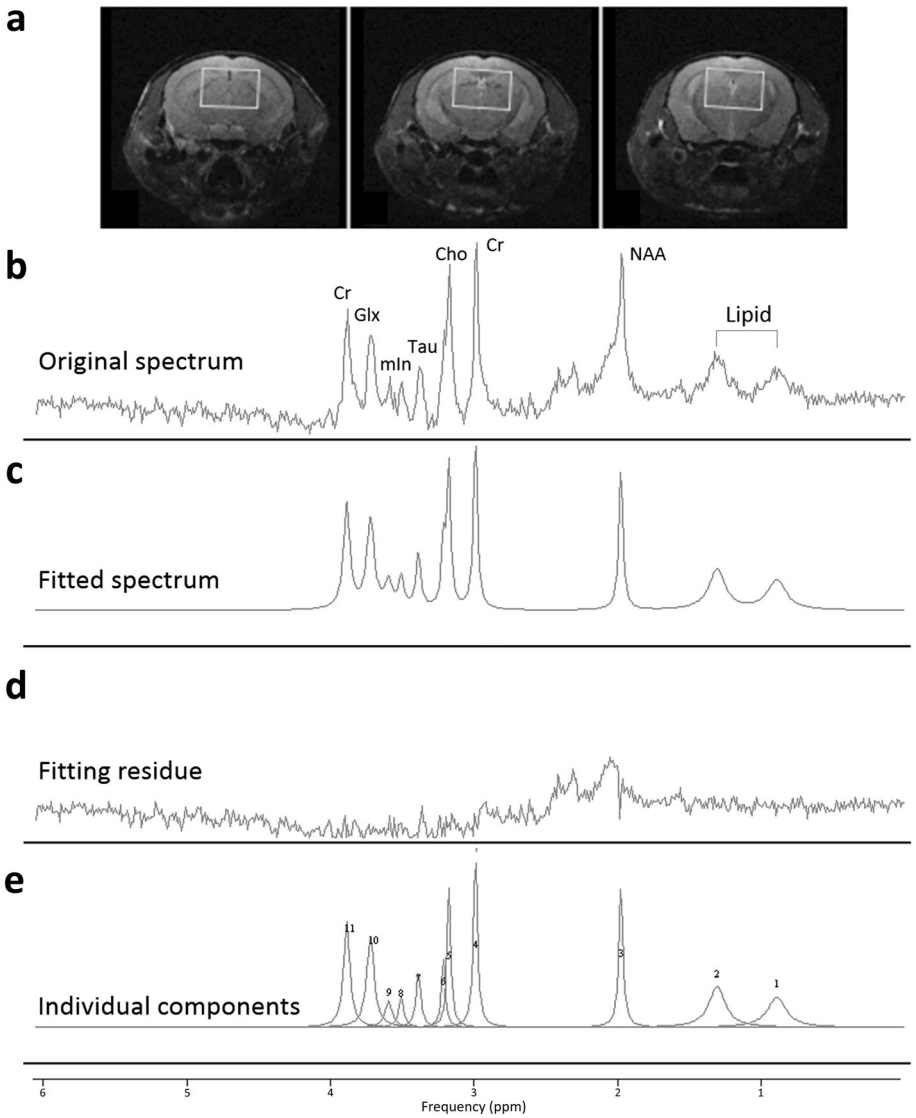

Fig. 1. **a** Example of a T2-weighted MRI scan of the brain in a C57BL/6 mouse from 27-month age group. MRS voxel location is overlaid with the T2w MRI (green boxes). **b** Single voxel proton MRS from the same mouse. **c** Fitting results from jMRUI. **d** Difference between original data and fitted spectrum (**b**–**c**). **e** Individual components are identified. NAA N-acetyl-aspartate, Cr creatine, Cho choline, Tau taurine, mIn myo-inositol, Glx glutamine + glutamate.

hippocampus and part of the thalamus. These spectra were analyzed with jMRUI to obtain ratios of concentrations of the major metabolites with respect to total creatine (Cr). Quality of fittings is demonstrated as individual components of peaks, combined model spectrum, as well as fitting residue as shown in Fig. 1.

Fig. 2 demonstrates MRS-measured brain metabolite concentrations normalized to total creatine for all age groups. Compared with the 4-month-old group, taurine levels dropped significantly only at 27 months of age ($p = 0.03$), but not at 18 months. However, a significant decrease of N-acetyl-aspartate (NAA) in the brain is observed at both 18 and 27 months ($p = 0.03$ and $p = 0.02$ respectively).

These findings suggest a sequential deterioration from young-old (18 months) to old-old (27 months) age by neuronal cell death, evidenced by the NAA decrease, followed by decreased neurogenesis, inadequately replacing the lost neurons reflected by the taurine decrease (Fig. 3).

Other metabolites, including total lipid, total choline, myo-inositol, as well as a mixture of glutamine and glutamate, did not show any statistical differences across all age groups (Fig. 2).

Correlation of Hippocampal Volume with Taurine in the Brain

Hippocampal volume, obtained using high-resolution T2w MRI, obviously decreased with age. We documented the loss of hippocampal volume by as much as 21 %, comparing 27-month with 4- month-old groups. In addition, MRS-measured taurine levels correlated well with hippocampal volume (Fig. 4, $R = 0.95$).

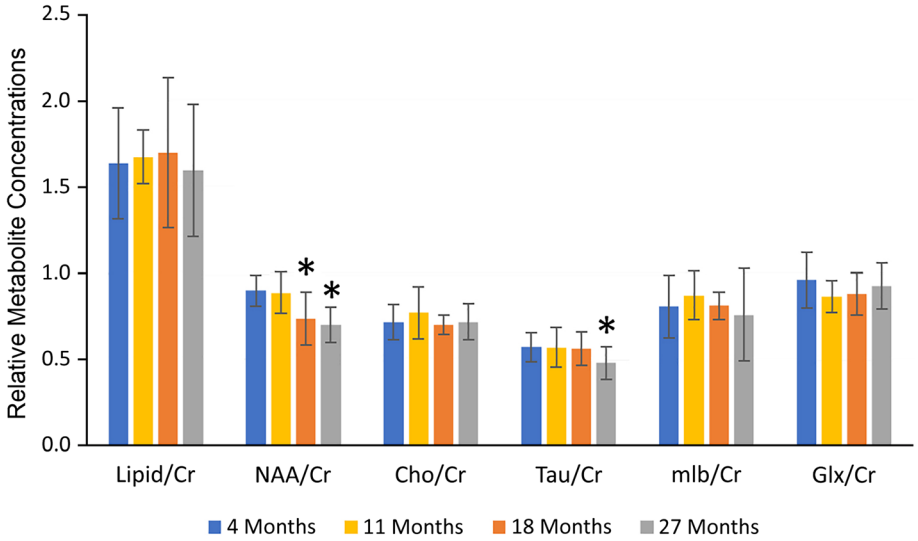

Fig. 2. MRS-measured brain metabolite concentrations in various age groups of C57BL mice. *Indicates that the specific age group differs statistically compared with the corresponding 4-month-old group ($p < 0.05$). NAA N-acetyl-aspartate, Cr creatine, Cho choline, Tau taurine, mIn myo-inositol, Glx glutamine + glutamate.

Discussion

Statistically significant reduction of brain N-acetyl-aspartate (NAA) in C57BL/6 mice starting at 18 months of age, observed in this study, is consistent with previously reported NAA decrease at this age (16–18 months) [20]. NAA, widely accepted as a neuronal biomarker, is used as an indicator of disease progression in human AD patients [21–23]. However, unlike human AD, in which most studies consistently report elevated glial marker myo-inositol (mIn) [21, 23, 24], no change in mIn levels is observed in the present study using C57BL wild-type mice; this lack of change of mIn during aging in C57BL mice is in accordance with a published study [20]. Our finding also indicates no significant increase of brain gliosis in elderly C57BL wild-type mice. Interestingly, dramatically increased mIn is found in APP-PS1 AD mice as early as 13 months and shows the most profound increase after 20 months [20]. The authors attribute this mIn increase to microglial activation in APP-PS1 mice and claim that this animal strain models human AD brain changes better than other AD models, such as APP and PS2APP [20].

Taurine is reported to play the role of osmoregulator in the brain [25]. Taurine supplementation also shows neuroprotective effects on the aging brain [5]. On the other hand, taurine levels related to aging have not been studied thoroughly in AD, either in clinical or preclinical settings. One of the main reasons is the lack of a method to accurately quantify taurine *in vivo* changes in the brain, and taurine does not change dramatically after early brain development and during adulthood. Indeed, Chaney et al. [16] found no differences in the taurine levels measured by MRS in C57BL wild-type mice aged 6–18 months, which is consistent with our findings. To our knowledge, the current study is the first one to measure *in vivo* brain metabolites using advanced

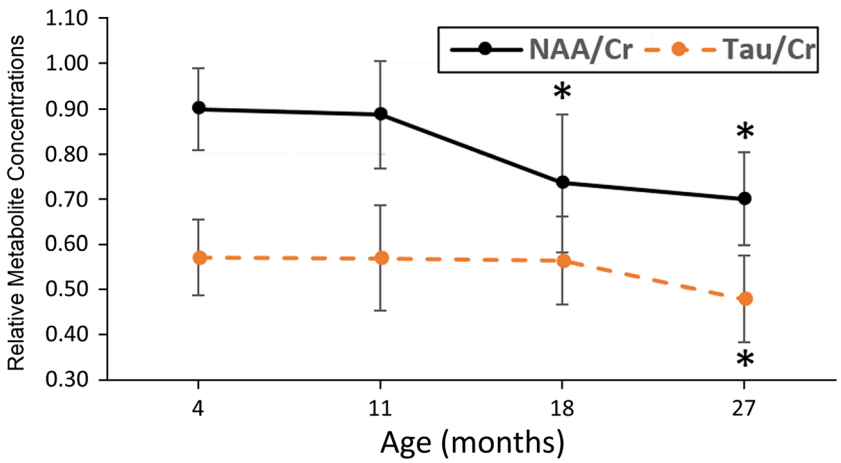

Fig. 3. Comparison of neuronal marker NAA with taurine in the brain over time demonstrated an earlier onset of NAA decrease (18 months) than taurine (27 months). *Indicates that the specific age group differs significantly from the 4-month-old group ($p < 0.05$).

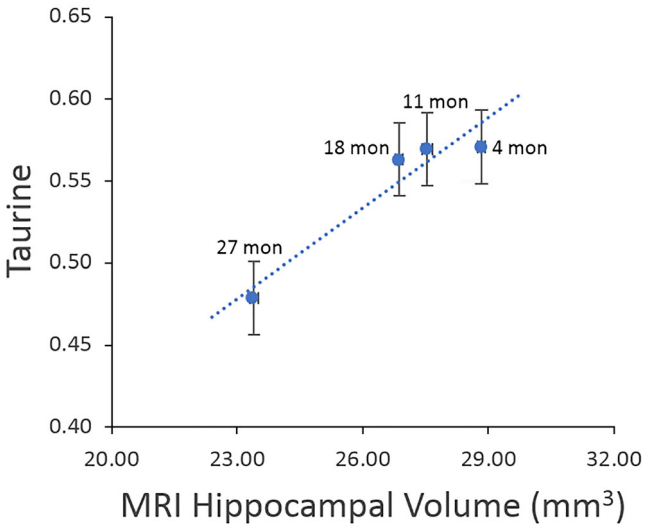

Fig. 4. Correlation of taurine level and the hippocampal volume based on age groups.

MRS methods in aging C57BL mice, covering the entire spectrum of 4 to 27 months of age. In the present study, the taurine level did not change significantly before 18 months of age but dropped significantly at 27 months. The later decrease of taurine compared with neuronal loss (NAA drops at around 18 months) indicates a possible connection between the two metabolites (Fig. 3). Indeed, a recent study by Gebara et al. [9] reports that taurine increases the survival of newborn neurons, resulting in a net increase in adult neurogenesis in middle-aged mice. The time difference between the onset of neuronal loss and taurine decrease may reveal a window of therapeutic opportunity. Taurine supplementation at young-old age should be considered when neurogenesis is needed to replace the loss of neurons.

Hippocampal volume decrease is a well-known hallmark of neurodegeneration-associated cognitive decline during aging [26]. This decrease is associated with greater severity of dementia in patients with Alzheimer's disease (AD) [27, 28]. Decreased hippocampal volume is correlated with neuronal marker NAA in a triple transgene AD mouse model [17], and also in *tau* transgenic mice (rTg4510) [29]. Our finding of a strong correlation between taurine level and hippocampal volume (Fig. 4) suggests that taurine may be related to decreased hippocampal volume, and thus an indicator of neurodegeneration.

In the current study, we used creatine from the same MRS voxel as an internal reference to obtain relative metabolite concentrations. We realize the limitation of the approach of assuming creatine being relatively stable and not changing significantly over age in C57BL mice brain. This is supported by the fact that creatine has been reported to be independent of age both in C57BL mice brain [17] and in the human brain [30]. When tested using internal water as reference, we found that the in-group variability and between-group variability become much higher than using creatine as reference (data not shown). This further implies that water from the same voxel is not a reliable reference to normalize metabolites in the brain. Indeed, the study had shown that under typically varying conditions *in vivo*, concentrations relative to total creatine peak are generally more accurate than absolute concentrations [31].

Conclusion

The current study establishes an MRS methodology for quantifying age-dependent changes in NAA and taurine distribution in the mouse brain during normal aging, thus suggesting that this preclinical study could easily be translated to unveil changes in the elderly human brain in these two metabolites. Most importantly, the loss of NAA in young-old (18 months) mice precedes the reduction of taurine in old-old (27 months) animals, suggesting that these two metabolites' decrease could be powerful biomarkers for decreased hippocampal volume during aging. In summary, decreased hippocampal volume, indicated by decreased levels of these two metabolites, could be powerful real-time biomarkers for neurodegeneration in aging humans, as well as for dementia progression in cognitive decline in Alzheimer's disease.

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Compliance with Ethical Standards. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Conflict of Interest

Dr. Eugenia Wang is on entrepreneurial leave with 51 % of her effort employed by the Advanced Genomic Technology, LLC; Dr. Christine Akimana is funded by Gheens Endowment Funds through the University of Louisville; Dr. Chin K. Ng and Dr. Mingming Zhu have no conflict of interest to disclose.

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