Changes of hippocampal N-acetyl aspartate and volume in Alzheimer's disease

A proton MR spectroscopic imaging and MRI study

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Article abstract—Hippocampal atrophy detected by MRI is a prominent feature of early Alzheimer's disease (AD), but it is likely that MRI underestimates the degree of hippocampal neuron loss, because reactive gliosis attenuates atrophy. We tested the hypothesis that hippocampal N-acetyl aspartate (NAA; a neuronal marker) and volume used together provide greater discrimination between AD and normal elderly than does either measure alone. We used proton MR spectroscopic imaging (^{1}H MRSI) and tissue segmented and volumetric MR images to measure atrophy-corrected hippocampal NAA and volumes in 12 AD patients (mild to moderate severity) and 17 control subjects of comparable age. In AD, atrophy-corrected NAA from the hippocampal region was reduced by 15.5% on the right and 16.2% on the left (both $p < 0.003$), and hippocampal volumes were smaller by 20.1% ($p < 0.003$) on the right and 21.8% ($p < 0.001$) on the left when compared with control subjects. The NAA reductions and volume losses made independent contributions to the discrimination of AD patients from control subjects. When used separately, neither hippocampal NAA nor volume achieved to classify correctly AD patients better than 80%. When used together, however, the two measures correctly classified 90% of AD patients and 94% of control subjects. In conclusion, hippocampal NAA measured by ^{1}H MRSI combined with quantitative measurements of hippocampal atrophy by MRI may improve diagnosis of AD.

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Neuropathologic studies of brains from patients with Alzheimer's disease (AD) demonstrate neuritic plaques and neurofibrillary lesions, accompanied by neuron loss and gliosis. Neuron loss results in atrophy of the cerebral cortex and other neuron-containing structures, including the hippocampal formation. Hippocampal atrophy in AD has been associated with impairment of declarative memory functions that are characteristic symptoms of AD.[1] Previous studies on AD patients using MRI found atrophy of the hippocampus.[2] Although, an initial MRI study of hippocampal atrophy reported a complete separation of AD patients from healthy elderly,[3] other studies of larger numbers of subjects showed some overlap between these groups.[4,5] One possible reason for this overlap is that to the degree neurons are replaced by glial cells, tissue atrophy in AD is attenuated.

Proton magnetic resonance spectroscopy (^{1}H MRS) and ^{1}H MRS imaging (^{1}H MRSI) detect important cerebral metabolites in vivo, including the amino acid N-acetyl aspartate (NAA), which is specifically located in neurons and absent in glia.[6] In the presence of reactive gliosis, NAA measured by ^{1}H MRSI may be a more sensitive marker of neuron loss than atrophy measured by MRI. We[7,8] and others[9-15] have documented a decrease of NAA in various brain regions of AD patients. Furthermore, MacKay et al.,[16] who found reduced NAA in the supraventricular cortex of AD patients, showed that these NAA reductions were to some degree independent from MRI changes, and demonstrated that when NAA measures were combined with measurements of ventricular volumes, AD patients and control subjects could be better classified than with either measure alone. Finally, a recent study[17] using multislice ^{1}H MRSI demonstrated a regional pattern of NAA reductions in AD involving the frontal, temporal, and parietal cortices, which is consistent with the known distribution of AD pathology.

Initially, technical considerations complicated ^{1}H MRSI measurements from the hippocampal region. But we[18] and others[19,20] have used ^{1}H MRSI to study changes of hippocampal NAA at the side of the sei-

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zure focus in patients with temporal lobe epilepsy. Block et al.[21] recently measured decreased NAA or increased choline (Cho) in the hippocampus of AD patients. However, quantitative results were not obtained and it remained unclear to what extent these metabolite changes were simply an artifact of MRSI partial volume effects, including variations in the tissue composition of MRSI voxels. Therefore, the goals of this study were (1) to test the hypothesis that NAA measured by ^{1}H MRSI is lower in the hippocampus of AD patients compared with control subjects, (2) to demonstrate that these NAA reductions are not an artifact of partial volume effects, (3) to confirm previous reports that hippocampal volume measured by MRI is smaller in AD patients than in control subjects, and (4) to test the hypothesis that hippocampal NAA and volume contribute independent information regarding AD pathology that, when used together, provide greater discrimination between AD and control subjects than either measure alone.

Methods. *Patients and control subjects.* Twelve patients (mean age ± SD, 74.3 ± 8.0 years; range, 55 to 82 years; eight women and four men) with the diagnosis of AD (nine probable and three possible) according to the NINCDS/ADRDA criteria[22] and with a mild or moderate level of dementia severity (Mini-Mental State Examination [MMSE][23] scores >12), and 17 cognitively normal subjects of similar age and sex distribution were studied. The diagnosis of possible AD for three patients was based on the observation that at the time of the evaluation two of these patients had thyroid problems and another suspected neurosyphilis. All subjects were recruited from the University of California (UC) San Francisco and the UC Davis Alzheimer Centers, were examined by a neurologist, and had the standard battery of blood and neuropsychological tests at the Centers. The 17 control subjects had an evaluation similar to that of the AD patients and were judged to be cognitively normal and functioning. None of the patients or control subjects had evidence of stroke, cortical or subcortical infarctions, or other major abnormalities on MRI, which were read by a neuroradiologist (D.N.). The protocol was approved by the Committee on Human Research at UC San Francisco, and all subjects or their legal guardians gave written informed consent before participating in the study. The 1-hour-long combined MRI/MRSI examination was completed by 10 AD patients and all control subjects. Two other patients requested to be taken out of the magnet before MRI/MRSI was completed. One of these patients finished MRI for segmentation and voluming, while the other had MRI for segmentation only. MRSI from one of the control subjects was not considered for analysis because of poor spectral quality, whereas MRI from this subject was satisfactory.

MRI/MRSI examinations. All studies were performed on a 1.5-T Magnetom VISION system (Siemens Inc., Iselin, NJ) equipped with a standard quadrature head coil. To minimize motion of the subject's head, a vacuum-molded head holder (Vac-Pac, Olympic Medical, Seattle, WA) was employed to restrict head movements. The MRI protocol consisted of sagittal T1-weighted localizer scans, oblique axial double spin-echo (DSE) scans angulated parallel to the optic nerve as seen in the sagittal plane, and a volumetric (three-dimensional [3D]) magnetization prepared rapid gradient echo (MP-RAGE) acquisition angulated perpendicular to the DSE image planes yielding T1-weighted coronal images estimated to be orthogonal to the long axis of the hippocampus. The measurement parameters of DSE were $TR/TE_1/TE_2$ = 3,000/20/80 ms, 1.0 × 1.4 mm^2 resolution, and 48 to 51 contiguous, 3-mm-thick slices covering the entire brain from the inferior cerebellum to the vertex. The measurement parameters of 3D MP-RAGE were TR/TI/TE = 10/250/4 ms, flip angle = 15 deg; 1.0 × 1.0 mm^2 resolution, and 1.4-mm-thick partitions.

^{1}H MRSI data sets were acquired using a spin-echo two-dimensional MRSI sequence at TR/TE = 1,800/135 ms with preselection of a region of interest (PRESS volume), requiring a total acquisition time of about 13 minutes. The PRESS volume was angulated parallel to the long axis of the hippocampi as seen from the sagittal scout images and positioned on the axial plane to cover both hippocampi in their entire length and adjacent sections of the midbrain and the temporal lobes. The MRSI field of view was 210 × 210 mm^2 and was sampled using a circular k-space scheme equivalent to a maximum of 24 × 24 phase encoding steps,[24] resulting in a nominal voxel resolution of 1.1 mL. The spectral sweep width was 1,000 Hz. Figure 1 shows axial T1-weighted MR images from an 80-year-old AD patient (figure 1A) and a 74-year-old control subject (figure 1B) at the position of the hippocampus and the corresponding NAA images, restricted to the sensitive area of the PRESS volume. Also shown are representative ^{1}H MR spectra selected from the hippocampal body of the AD patient and control subject (location and approximate size of the MRSI voxel is indicated by a circle in the corresponding MRI). The three prominent resonances in the ^{1}H MR spectrum are from NAA, and Cho- and creatine (Cr)-containing compounds.

MRI segmentation and "voluming." Tissue segmentation on the whole brain and voluming of the hippocampus was performed using software developed in house (GF). The semiautomated segmentation software uses both T1-weighted MP-RAGE and T2-weighted spin-echo images. The first-pass segmentation procedure automatically removes the skull and meninges from the images, coregisters the 3D T1-weighted images to each of the two interleaves of the spin-echo images using Wood's algorithm,[25] performs 3D inhomogeneity correction using a digital filter,[26] and performs segmentation on the whole brain using K-Means cluster analysis via the SAS FASTCLUS procedure.[27] For the cluster analysis, seeds for each tissue category, i.e., gray matter [GM], white matter [WM], and CSF are first defined based on regions around the peaks in the T1 pixel intensity histogram. These regions represent conservative estimates of the appropriate tissue category. If desired, the initial process is followed by manual editing of the data to separate cortical from subcortical GM, ventricular CSF from sulcal CSF, and to reclassify pixels incorrectly classified as GM into a category of WM signal hyperintensity. The number of pixels for each tissue category is expressed as a percentage of total intracranial volume (TIV), which equals the total number of pixels.

Quantitative estimates of the volumes of the right and left hippocampus were obtained using coronal T1-weighted MP-RAGE images, resliced perpendicular to the long axis

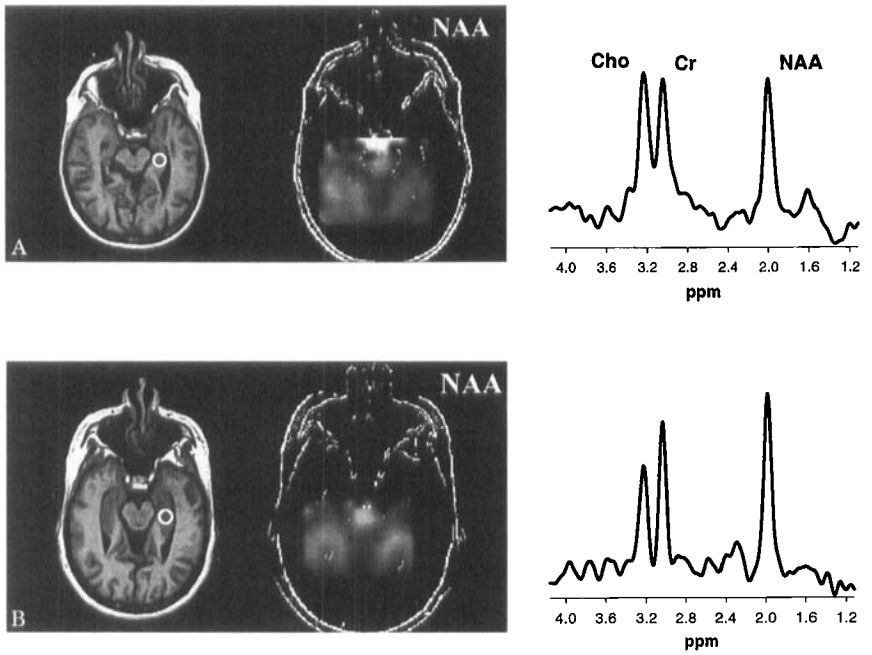

Figure 1. Axial MR images from an 80-year-old Alzheimer's disease (AD) patient (A) and a 74-year-old control subject (B) at the position of the hippocampus, and the corresponding N-acetyl aspartate (NAA) images from this region, restricted to the sensitive area of the preselected region of interest (i.e., PRESS volume). Contours of the MRI are superimposed on the NAA image for better anatomic reference. Note that MRI and NAA images have different field of views. Also shown are representative proton MR spectra selected from the hippocampal body (location indicated by a circle in the MRI). Reduction of NAA in the AD patient with respect to the control subject becomes apparent when the NAA peak intensity in each spectrum is compared with creatine (CR). Cho = choline.

of the hippocampus. Boundaries of the hippocampus were drawn following specifically the guidelines of Watson et al.[28] The areas of all regions of interest were then automatically calculated and the total volume derived by multiplying that value by the slice thickness. All volume measurements were performed by the same rater (D.A.), who was blinded to the subject's diagnosis. To evaluate reliability of volume measurements, data from 16 arbitrarily selected subjects were independently volumed a second time by another experienced operator. The correlation coefficient between these two measurements was >0.92. To adjust for interindividual variations in head size, each subject's hippocampal volume, $HV^{(s)}$, was normalized to his/her intracranial volume index, $TIV/TIV^{(s)}$, where TIV represents the mean total intracranial volume of the control group and $TIV^{(s)}$ is each subject's total intracranial volume, computed from the segmented images. This procedure maintains the dimension of the normalized hippocampal volumes, in contrast to the normalization by $1/TIV^{(s)}$, and is justified as long as the mean TIV values of the study groups are not significantly different. In this population, patients and control subjects had comparable TIV values (difference <1%, $p > 0.96$ by ANOVA), although others[29] found a modest correlation between reduced TIV sizes and deficits of some cognitive functions.

MRSI analysis. After acquisition, the ^{1}H MRSI data were zero filled to a rectangular matrix of 32 × 32 × 1,024 points, fourier transformed, and phase- and baseline-corrected using software developed in house.[30] Four-Hertz gaussian line broadening was used in the spectral direction, and mild gaussian apodization was applied along the spatial directions to reduce Gibbs' ringing effects, resulting in an effective volume of the MRSI voxels of approximately 1.6 mL. Voxels were selected from the head, body, and tail of the right and left hippocampus, and the resonances from NAA, Cr, and Cho were curve fitted using NMR1 software (New Research Methods Inc., Syracuse, NY). To estimate concentrations in millimoles per liter (mM), integral values of the metabolite resonances were referenced to values obtained from a head-sized phantom, including corrections for coil loading, receiver gain, and metabolite T1 and T2 relaxation rates, using T1 and T2 values from a previously reported ^{1}H MRS study in healthy elderly.[11] It was assumed that metabolite relaxation rates were the same for AD patients and normal elderly, and for GM and WM.

To obtain atrophy-corrected metabolite intensities and to verify that metabolic changes were not an artifact of partial voluming, all MRSI data in this study were analyzed for variations of voxel compositions in terms of GM, WM, and CSF using software developed in house (F.E., G.F.). This computation used the tissue segmented images coregistered with the MRSI data to estimate the composition of MRSI voxels and was performed with consideration of the MRSI point spread function, chemical shift displacement effects, and signal sensitivity across the PRESS volume, which was determined experimentally on a head-sized phantom. Assuming that no metabolites are observed in CSF, the composition of each MRSI voxel can be characterized by two parameters: tissue content, $\rho = (GM + WM)$, and gray matter index, $f = GM/(GM + WM)$. Then, atrophy-corrected metabolite intensities (i.e., of NAA) were computed according to

$$NAA = NAA'' \times 1/\rho$$

with NAA'' being the uncorrected intensity, and f as the covariate for the statistical analysis to determine the extent to which the tissue composition contributed to metabolite differences.

Typically, MRSI spectra obtained from the hippocampal body exhibited a better spectral resolution than spectra from the hippocampal head, which often were degraded from poor B_0-field homogeneities in this region and furthermore contained more GM than voxels from the tail, reflecting the brain anatomy in this region. We concluded that MR spectra from the hippocampal body would best be suited to measure metabolic changes in the hippocampus

Table 1 *Clinical characteristics of patients with Alzheimer's disease (AD) and control subjects*

Characteristic	AD	Control
No. of subjects	12	17
Mean age (y)*	74.1 ± 8.3	72.2 ± 5.6
Age range (y)	54–81	61–85
Women/men	8/4	14/3
MMSE score*	18.4 ± 5.2	29.1 ± 0.8
MMSE score range	12–28	28–30
Mean duration of symptoms (y)*	4.2 ± 1.8	N/A

* Mean value ± SD.

MMSE = Mini-Mental State Examination; N/A = not applicable.

and therefore used these spectra exclusively for further analysis.

Statistical analysis. Repeated measures ANOVA (SAS, SAS Institute Inc., Cary, NC) was used to test group differences of hippocampal volumes and gross atrophy measures. ANCOVA (BMDP, Statistical Solutions Inc., Cork, Ireland) with tissue composition f as the covariate was used to test, by group and by side, differences of metabolite concentrations in the right and left hippocampus. To determine the extent that hippocampal NAA and volume were independent of each other, NAA was used as the independent variable and volume as covariate, and vice versa, to determine group membership by ANCOVA. Results are expressed as mean ± SE unless otherwise indicated. The level of significance for differences is $p < 0.05$.

Results. *Demographics.* The demographic data are summarized in table 1. Patients and elderly control subjects were comparable in age ($p > 0.5$ ANOVA) and had a similar gender distribution (67% and 82% women in the patient and control groups, respectively). The AD patients had a mean MMSE score of 18.4 ± 5.2 (SD) with a range from 12 to 28, and an average duration of symptoms of 4.2 ± 1.8 (SD) years. Elderly control subjects had MMSE scores of at least 28 or better.

MR spectroscopic imaging. Table 2 lists the results of atrophy-corrected NAA, Cr, and Cho, and the ratios of NAA to Cr and NAA to Cho from the left and right hippocampus in AD patients and control subjects. Also listed are ρ (in percent of total voxel content) and f, revealing significant differences in the composition of MRSI voxels between the two groups. After correcting for atrophy by ρ, NAA from the right and left hippocampus in AD was reduced by 15.5% and by 16.2% ($p < 0.003$) respectively compared with control subjects. Variations of tissue composition did not contribute significantly to the differences ($p > 0.98$). Furthermore, both NAA/Cr and NAA/Cho were significantly reduced ($p < 0.02$ and $p < 0.03$ respectively) in AD patients compared with elderly control subjects, providing additional evidence that reductions of NAA in AD cannot be entirely attributed to atrophy. The con-

Table 2 *Atrophy-corrected metabolite concentrations of N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr), and NAA/Cr and NAA/Cho ratios from the right and left hippocampus in Alzheimer's disease (AD) patients and control subjects*

Variables	AD	Control	Difference (%)	p Value
NAA (mM)				
Right	7.67 ± 0.2	9.08 ± 0.2	−15.5	<0.003
Left	7.80 ± 0.3	9.31 ± 0.3	−16.2	
Cho (mM)				
Right	1.71 ± 0.1	1.79 ± 0.2	−4.5	NS
Left	1.97 ± 0.2	1.90 ± 0.1	3.6	
Cr (mM)				
Right	6.91 ± 0.4	7.47 ± 0.4	−7.5	NS
Left	8.24 ± 0.4	7.82 ± 0.4	5.4	
NAA/Cr				
Right	1.42 ± 0.07	1.59 ± 0.06	−10.7	<0.02
Left	1.25 ± 0.06	1.56 ± 0.06	−19.8	
NAA/Cho				
Right	1.28 ± 0.07	1.48 ± 0.09	−13.4	<0.03
Left	1.21 ± 0.09	1.46 ± 0.09	−17.4	
Tissue content, ρ (%)				
Right	85 ± 2	96 ± 1	−11.4	<0.01
Left	88 ± 2	97 ± 1	−9.3	
Gray matter index, f				
Right	0.44 ± 0.02	0.52 ± 0.02	−15.3	0.01
Left	0.59 ± 0.04	0.56 ± 0.03	5.3	>0.5

* Also listed are tissue content, ρ (in percent of the MR spectroscopic imaging [MRSI] voxel volume), and gray matter index, f, of the MRSI voxels positioned at the right and left hippocampus, characterizing MRSI partial volume effects.

Table 3 *Normalized hippocampal volumes (HP-volume) and volumes of cortical and subcortical gray matter (GM), white matter (WM), sulcal and ventricular CSF as a percent of the total intracranial volume (TIV) in Alzheimer's disease (AD) patients and control subjects, and mean TIV of AD patients and control subjects*

Variables	AD	Control	Difference	*p* Value
HP-volume (mm^3)				
Right	2,416 ± 141	3,021 ± 111	−20.1	0.003
Left	2,364 ± 104	3,025 ± 99	−21.8	0.001
Ventricular CSF (%)	3.4 ± 0.3	2.5 ± 0.2	34.1	0.03
Sulcal CSF (%)	21.8 ± 1.2	17.8 ± 0.4	22.8	0.01
WM (%)	33.4 ± 0.8	36.1 ± 0.5	−7.3	0.01
Cortical GM (%)	39.5 ± 1.0	42.1 ± 0.5	−6.1	0.03
Subcortical GM (%)	1.1 ± 0.08	1.2 ± 0.08	−5.9	NS
TIV (cm^3)	1,344 ± 31	1,349 ± 46	−0.4	0.96

NS = not significant.

centrations of hippocampal Cr and Cho were not significantly different between AD patients and control subjects. In both groups, NAA, Cr, and Cho concentrations were not significantly higher in the left than in the right hippocampus ($p > 0.7$). ρ reveals that MRSI voxels from AD patients contained on average 10% less tissue than control subjects, reflecting the increased atrophy in AD patients. Accordingly, the difference of hippocampal NAA between the groups without atrophy correction (which reflects both NAA and volume changes) was about 40% larger than with correction of atrophy. This emphasizes the importance of correcting MRS and MRSI data for partial volume effects.

MRI voluming and segmentation. Table 3 lists the normalized hippocampal volumes in AD patients and elderly control subjects, as well as percent volumes of cortical and subcortical GM, WM, and ventricular and sulcal CSF. As expected, AD patients had smaller hippocampi on both sides than control subjects (on the right, 20.1% [p = 0.003], on the left, 21.8% [p <0.001]), and the right hippocampus was slightly larger (≈2%) than the left, but this difference was not statistically significant ($p > 0.5$). In AD patients the ventricular and sulcal CSF volumes were enlarged by 34.1% ($p = 0.03$) and by 22.8% ($p > 0.01$) respectively when compared with controls, and cortical GM and WM were reduced by 6.1% ($p = 0.03$) and by 7.3% ($p < 0.01$) respectively. In contrast, subcortical GM ($p > 0.6$) and total intracranial volume ($p > 0.96$) were not significantly different between AD patients and control subjects.

Combinations of MRSI and MRI measures. Figure 2 depicts the distributions of hippocampal NAA, hippocampal volume, and percent ventricular size in AD patients and control subjects, separated by gender. It can be seen that no measure alone achieves a complete separation of AD patients and control subjects. To explore whether hippocampal NAA and volume provide complementary information that may aid group classification, we tested to what extent these two measures are independent of each other. After controlling for group differences in hippocampal volumes by ANCOVA, significant differences of hippocampal NAA between the groups were still present (p

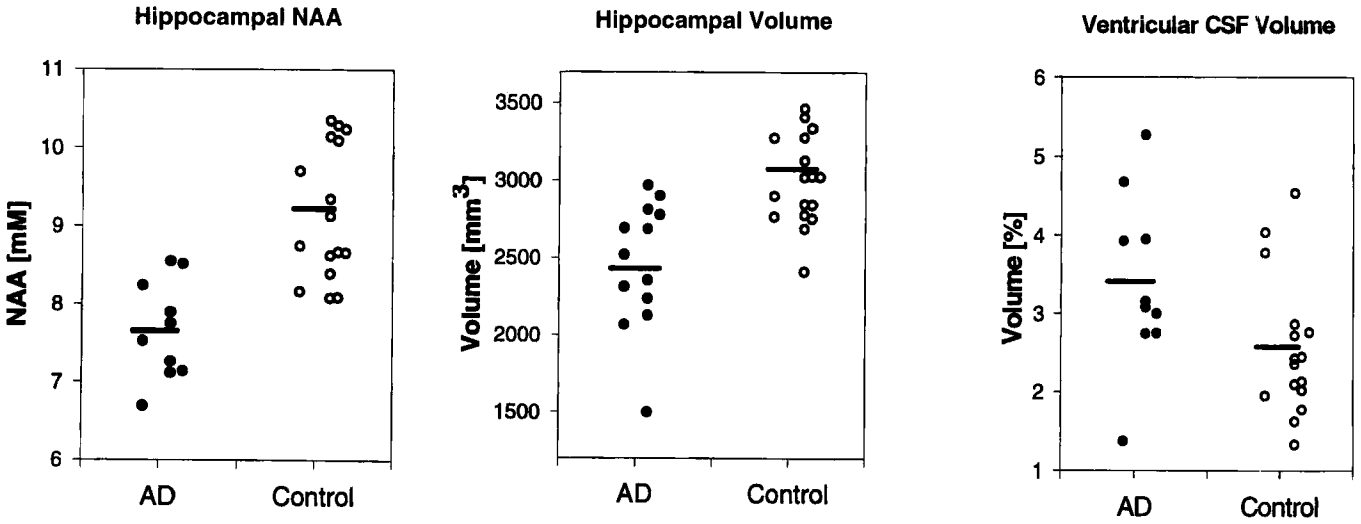

Figure 2. Distribution of atrophy-corrected hippocampal N-acetyl aspartate (NAA), normalized hippocampal volume, and percentage ventricular volume from Alzheimer's disease (AD) patients (●) and control subjects (○). NAA and volume are mean values of the right and left hippocampus. Values from male and female subjects are displayed in separate columns for each study group with the left column showing the values from the males. The group mean values are indicated by a bar (—).

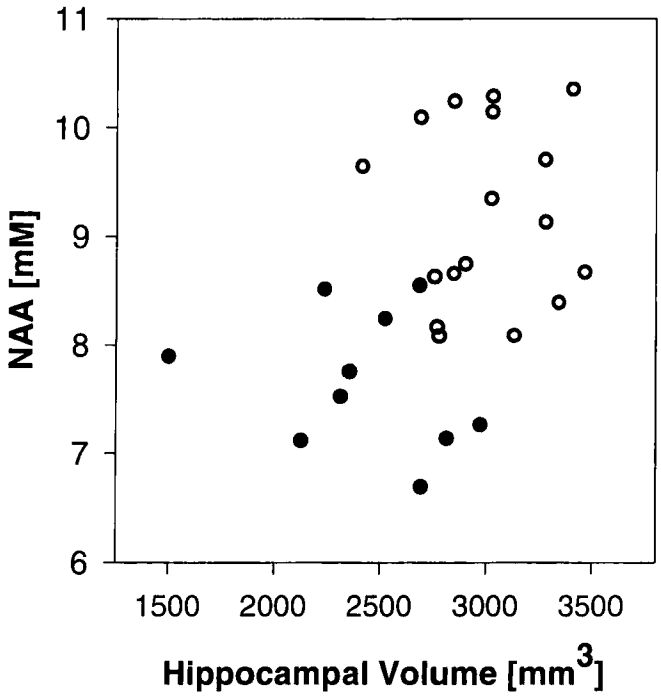

Figure 3. Atrophy-corrected hippocampal N-acetyl aspartate (NAA) as a function of normalized hippocampal volume from each of the 10 Alzheimer's disease patients (●) and 16 control subjects (○) with complete MRI/MR spectroscopic imaging examination. NAA and volume are mean values of the right and left hippocampus.

<0.002). Similarly, after controlling for group differences in hippocampal NAA, volume losses in AD remained significant (p <0.001). Taken together, these results reveal that hippocampal NAA and volume each provide independent information relevant to the discrimination of AD patients from control subjects. Neither NAA, nor volume, nor the combination of both (NAA × volume) could distinguish possible from probable AD (p > 0.7 by ANOVA). This suggests that the MRI and MRSI differences between AD patients and control subjects were not skewed by including the patients with possible AD. Finally, we tested whether the discrimination between AD patients and control subjects by the hippocampal NAA × volume index remains significant after adjusting for variations of age and dementia severity (as measured by MMSE) using ANCOVA. We found that neither age (p = 0.27) nor MMSE (p = 0.96) contributed significantly to the difference of the NAA × volume index between the groups, suggesting that the index is to a large extent independent from the patients' age and dementia severity.

Figure 3 shows the distribution of atrophy-corrected hippocampal NAA (right and left averaged) from each AD patient and control subject as a function of his/her hippocampal volume (right and left averaged). To determine the classification power of hippocampal NAA and volume when combined, we performed a stepwise linear discriminant analysis with hippocampal NAA being the first variable entered. Alone, hippocampal NAA correctly classified 80% (8 of 10) of the AD patients and 75% (12 of 16) of the control subjects. When hippocampal volume was entered, the two measures combined increased classification of AD patients and control subjects to 90% (9 of 10) and 94% (15 of 16) respectively. Entering in addition percent ventricular CSF did not improve the classification of AD patients and decreased that of control subjects.

Discussion. The major findings of this study were (1) atrophy-corrected NAA concentrations were significantly lower in the hippocampus of AD patients compared with control subjects of comparable age, (2) MRI-measured hippocampal volume was also lower in AD patients compared with control subjects, (3) reductions of atrophy-corrected hippocampal NAA were not an artifact of partial volume effects, and (4) reductions of hippocampal NAA and volume losses provided independent information regarding the discrimination of AD from control subjects, and when used together classified AD better than either measure alone. In conclusion, these findings suggest that measurement of hippocampal NAA by ^{1}H MRSI, when employed in conjunction with MRI, may provide improved discrimination between AD patients and control subjects, and ultimately may be useful to detect AD in the early stages of the disease.

The first major finding of this study was that NAA was reduced in the hippocampus of AD patients compared with control subjects of comparable age. This result is consistent with a previous ^{1}H MRSI study in the hippocampus of AD patients[21] that measured reduced hippocampal NAA/Cho and NAA/Cr, suggesting diminished NAA levels. However, the present experiment quantitatively measured absolute NAA in hippocampus and, furthermore, combined NAA and volume measurements to improve discrimination between AD patients and normal elderly. There is a considerable body of evidence concerning reduced NAA in the brain of AD patients. Kwo-On-Yuen et al.,[31] performing in vitro NMR measurements of AD brain tissue at post mortem demonstrated reduced NAA, consistent with neuron loss. Since then there have been several reports, including those from this laboratory,[7,8] indicating reduced NAA/Cr and/or NAA/Cho in AD, and a few quantitative ^{1}H MRS measurements documenting unambiguously lower NAA in AD.[11,13] Reports of reduced metabolite ratios have inferred that the reductions of NAA/Cr and NAA/Cho cannot simply be attributed to volume loss, because simple atrophy would result in reductions of both NAA as well as Cho and Cr. Most previous single-volume ^{1}H MRS and ^{1}H MRSI studies of AD have been performed in supraventricular brain regions involving frontal, parietal, and occipital cortex, and white matter. These studies did not include hippocampus, even though it is a major site of AD pathology, including neuron loss specifically.[32] This is probably because technical problems, including difficulties in obtaining sufficient homogeneity of the local static magnetic field and contamination from lipid resonances have complicated acquisition of spectra from medial temporal lobe and hippocampal regions. Our previous experience obtaining ^{1}H MRSI spectra from the hippocampal regions in patients

with temporal lobe epilepsy[18] indicated that ^{1}H MRSI of the hippocampus was feasible.

The second major finding of this report is that hippocampal volumes were reduced in AD patients compared with control subjects of comparable age. This finding is similar to several previous MRI reports, which have shown volume reductions in AD patients up to 48%[3] when compared with normal elderly. In contrast to the initial MRI studies of hippocampal atrophy, which reported a complete separation between AD patients and control subjects,[3] the present results are similar to those of others,[4,5] which found considerable overlap. One possible explanation for this overlap is that neuron loss in the hippocampus of AD patients is accompanied by reactive gliosis,[33] which attenuates tissue atrophy, resulting in an underestimation of volume loss by MRI and consequently in the failure to discriminate between AD patients and normal elderly.

The third major finding of this study is that reductions of hippocampal NAA corrected for atrophy are not an artifact of differences in tissue composition of the MRSI voxels between groups. In an earlier study MacKay et al.[16] from this laboratory, using a combined analysis of coregistered MRI and ^{1}H MRSI data, demonstrated that NAA differences measured in supraventricular regions of AD patients and control subjects existed independent of variations of the tissue characteristic in MRSI voxels. The current results demonstrate that this observation can be extended to the hippocampus. The current analysis was made possible by the development of semiautomated segmentation and voluming software with accurate coregistration of the MRI and ^{1}H MRSI data. There have been few attempts[34,35] to measure quantitatively metabolic changes by ^{1}H MRSI with consideration of partial volume effects. Aside from the previous report by MacKay et al.,[16] there have been no attempts to determine statistically the extent to which metabolic changes in AD are independent from variations of the tissue characteristic in MRSI voxels.

The most important finding of this study is that hippocampal NAA and volume provide independent information regarding the discrimination between AD patients and normal elderly. This result led us to attempt to use both measures to improve discrimination between AD patients and control subjects. Figure 3 depicts the distribution of hippocampal NAA as a function of hippocampal volume from each subject and demonstrates that the combination of the two measures provided better correct classification of AD patients and control subjects in this study population than either measure alone. However, this result does not imply clinical applications for the diagnosis of AD. Further studies on a larger population, especially with unselected subjects, are necessary to assess the diagnostic value of the NAA $\times$ volume index in comparison with that available with current clinical methods. In a strict sense, this can be achieved only with longitudinal studies that culminate in the pathologic confirmation of AD in each patient. Figure 3 also shows an overlap between patients and control subjects for both hippocampal NAA and volume measures. There are several explanations for this finding. Increasing neurofibrillary tangle burden[36] and neuronal loss[37] with age are commonly found in hippocampal regions of nondemented elderly individuals, which could explain the low NAA levels and small hippocampi of some control subjects. Another explanation for the overlap includes the possibility that the control group may have included individuals with preclinical AD. Finally, the lack of a complete separation between AD patients and control subjects could also be interpreted in the sense of a neurobiological continuum between normal aging and dementia, a view supported by findings of several recent studies.[14,38] Nevertheless, the finding of this study that hippocampal NAA and volume provide independent information regarding the discrimination between AD patients and control subjects supports our hypothesis that ^{1}H MRSI in combination with MRI may be helpful in providing improved diagnosis and early detection of AD.

There are several limitations to this study. First, the AD patients have not yet been followed to autopsy, so it is not absolutely certain that the patients have AD. Second, data from elderly patients with dementias due to causes other than AD (such as vascular dementia) were not included. Therefore, it is not clear whether the reduction of hippocampal NAA found in AD is specific for this condition. However, previous ^{1}H MRSI studies in AD and in vascular dementia found metabolic abnormalities in WM regions of vascular dementia but not in AD,[8,15] raising the possibility that ^{1}H MRSI may be useful to distinguish between these two forms of dementia.

Third, the major technical limitation of this study was that the spatial resolution of ^{1}H MRSI is coarse, and nonhippocampal structures were probably included within the MRSI voxel, especially other structures in the limbic lobe. However, these structures are also involved with AD. Furthermore, this ^{1}H MRSI study was restricted to the hippocampal region and did not obtain MR spectra from other areas of the brain, including the frontal, parietal, and temporal cortices, which are also affected by AD. Greater brain coverage can be accomplished by using multislice ^{1}H MRSI[39] instead of volume preselection methods as applied in this study. Recently, Tedeschi et al.[17] employed multislice ^{1}H MRSI to measure metabolite ratios from large sections of frontal, parietal, and temporal lobes and thalamus in AD, but not from mesial temporal lobe and hippocampus. This laboratory has also developed a version of multislice ^{1}H MRSI[40] that should be useful for the assessment of AD.

Fourth, increased levels of myoinositol inversely correlated with NAA changes have been reported in AD using single ^{1}H MRS at relatively short spin-echo times (TE <30 ms).[41] The current study was performed at TE = 135 ms and does not permit the

detection of resonances from myoinositol, which exhibits T2 values in the order of 60 ms or less.[11] Development of multislice ^{1}H MRSI with short spin-echo times to accommodate simultaneous measurements of NAA, myoinositol, and other metabolites are currently under development. Finally, we did not attempt to measure metabolite relaxation times T1 and T2 because of the prohibitively long duration of the data acquisition. Instead, we used T1 and T2 values for NAA, Cr, and Cho documented in a previous report of MRS in healthy elderly[11] and applied these values to obtain approximations for the metabolite concentrations in AD patients and control subjects. This analysis is limited in that it ignores the possibility of T1 and T2 alterations with regions and/or disease. To our knowledge, there is no evidence of abnormal T1 values in AD. Christiansen et al.[13] using single-volume MRS, reported prolonged T2 times for NAA in frontal WM of AD subjects compared with control subjects. If T2 for NAA was also prolonged in the hippocampus, the current measurements would have overestimated NAA in AD, and thus underestimated the differences with control subjects.

In conclusion, this report demonstrates reductions of volume-corrected NAA, a measure of neuronal density, in the hippocampus of patients with AD compared with control subjects of comparable age. These NAA reductions are statistically independent from hippocampal volume losses, and NAA taken together with volume provides better discrimination between AD patients and control subjects than either measure alone. These findings suggest that measurement of NAA by ^{1}H MRSI provides complementary information about loss or damage of neurons in AD that is not available from measurements of atrophy by MRI. Ultimately, ^{1}H MRSI together with MRI may be helpful in providing improved diagnosis and early detection of AD.

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An alphabetical 'WORLD'

A new version of an old test

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Article abstract—The Mini-Mental State Examination (MMSE) is a standardized test used by neurologists to screen patients for impaired cognition. Despite its ease of use, one major limitation of the MMSE is a possible ceiling effect or a lower sensitivity in patients with advanced education. Patients (n = 97) undergoing diagnostic neuropsychological testing also completed one subtest of the MMSE, the spelling of "world." In addition to its forward and backward spelling, patients were asked to reorder these letters in alphabetical sequence. Our preliminary data indicate that our modified WORLD test is a rapid and simple test to identify patients with cognitive impairment. When measured against the diagnosis of dementia as determined by neuropsychological testing, the modified WORLD test has a sensitivity of 85%, a specificity of 88%, and a positive predictability value of 95%. Other variables examined include patient age, sex, education, and cutoff scores on the Mattis Dementia Rating Scale.

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The Mini-Mental State Examination (MMSE) is a standard tool used by neurologists in clinical practice to rapidly detect cognitive impairment.[1] This test is a series of tasks that assess orientation, immediate and short-term recall, attention and calculation, language, and visual construction. The MMSE examines attention by asking patients to subtract serial 7's or spell "world" backwards. Both tasks have high diagnostic sensitivity but low specificity. The WORLD subtest is often favored because it de-emphasizes mathematical skills.

The major limitation of the WORLD test is a possible ceiling effect or a lower sensitivity in patients with advanced education. Improved sensitivity and specificity can be achieved by using more sophisticated neuropsychological tests (e.g., Wisconsin Card Sorting test),[2] but their methodologic or scoring complexities render them impractical as bedside screening procedures. To improve the accuracy of bedside cognitive testing, we modified the standard WORLD test by asking patients to reorder the letters in "world" in alphabetical sequence. We present the results of a pilot study to determine the validity of this simple and rapid test as a potential marker of cognitive dysfunction.

Methods. Consecutive physician-referred patients (n = 97) undergoing neuropsychological evaluation for possible dementia or depression completed a series of standard tests administered by one author (A.J.B.). These tests included the Mattis Dementia Rating Scale (DRS),[3] Boston Naming Test (BNT), Complex Ideation Test (CI),[4] Recipro-

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Changes of hippocampal N-acetyl aspartate and volume in Alzheimer's disease: A proton MR spectroscopic imaging and MRI study

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