In Vivo Brain Concentrations of N-Acetyl Compounds, Creatine, and Choline in Alzheimer Disease

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Background: Alzheimer disease (AD) and normal aging result in cortical gray matter volume deficits. The extent to which the remaining cortex is functionally compromised can be estimated in vivo with magnetic resonance spectroscopic imaging.

Objective: To assess the effects of age and dementia on gray matter and white matter concentrations of 3 metabolites visible in the proton spectrum: N-acetyl compounds, present only in living neurons; creatine plus phosphocreatine, reflecting high-energy phosphate metabolism; and choline, increasing with membrane synthesis and degradation.

Method: Fifteen healthy young individuals, 19 healthy elderly individuals, and 16 patients with AD underwent 3-dimensional magnetic resonance spectroscopic imaging and memory and language testing.

Results: Gray matter N-acetyl compound concentrations (signal intensity corrected for the amount of brain tissue contributing to the magnetic resonance spectroscopic imaging signal) was significantly reduced only in patients with AD, even though both the AD and elderly control groups had substantial gray matter volume deficits relative to the young control group. Both the healthy elderly and AD groups had abnormally high gray matter creatine plus phosphocreatine concentrations. Gray matter choline concentrations were higher in the elderly than the younger controls, and even higher in the AD group than in the elderly control group. Functional significance of these findings was supported by correlations between poorer performance on recognition memory tests and lower gray matter N-acetyl compounds in elderly controls and higher gray matter creatine plus phosphocreatine and choline concentrations in patients with AD.

Conclusion: Cortical gray matter volume deficits in patients with AD are accompanied by disease-related increases in gray matter choline concentrations suggestive of cellular degeneration and reduced N-acetyl compound concentrations, with possible effects on behavioral function.

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Alzheimer disease (AD) results in substantial loss of brain tissue. Healthy elderly individuals show an age-related gray matter volume decline in the neocortex (see Guttman et al) but their volume loss may not be accompanied by neuronal death. At issue is the functionality of the tissue remaining in the brains of healthy elderly patients and patients with AD.

In vivo proton magnetic resonance spectroscopic imaging (1H MRSI) allows examination of brain tissue integrity. The N-acetyl (NAC) peak is composed of several NAC compounds, including NAC aspartate, that are present only in living, mature neurons and not glia. Many studies have reported lower NAC signals in patients with AD compared with healthy elderly individuals; some, but not others, have reported age-related decline in brain NAC in healthy people. Most studies have employed single-voxel spectroscopy and have expressed NAC as a ratio of 1 or 2 other metabolites: creatine plus phosphocreatine (Cr), which reflects high-energy phosphate metabolism, and choline (Cho), which increases in signal intensity with membrane synthesis and degradation. Many proton spectroscopy studies assume that neither age nor AD seriously affects Cr or Cho levels and thus express data as ratios.

We developed an MRSI method to estimate absolute proton metabolite signal intensities in gray matter and white matter separately. Concentration, expressed...
as institutional signal intensity units per tissue volume, revealed more NAc in gray matter than white matter, consistent with many, but not all, studies. Despite significant gray matter volume deficits in elderly healthy individuals, the young and elderly groups had equivalent concentrations of NAc in gray matter and white matter. By contrast, Cr and Cho concentrations demonstrated significant age effects. Cho concentrations were lower in gray matter in older controls; Cr concentrations were greater in gray matter and white matter in older subjects. These observations draw into question the use of Cr and Cho as appropriate referents for determining NAc concentration.

We applied our MRSI method to patients with AD and compared their results with those from our study of normal aging. We expected the patients with AD, unlike the healthy elderly controls, to have abnormally low NAc concentrations in gray matter and white matter.
Structural Image Acquisition

A midsagittal, gradient-recalled echo image was used to compute slice positions with 0.3-mm accuracy for all 3 scans in this protocol (anatomical, fieldmap, and MRSI). Anatomic images were acquired with an axial fast-spin echo protocol (TR, 3000 milliseconds; and TE, 20/80 milliseconds; echo train length, 8; 3-mm skip, 0.2 mm; 256 × 256 pixels matrix; field of view, 24 cm; number of excitations, 1; and time, 3 minutes 18 seconds). Sixteen slices were collected, the most inferior slice beginning at the anteroposterior commissure line, corresponding to the 8 middle spectroscopic slices and providing 2 anatomical slices for each MRSI slice. Twelve of these high-resolution images, corresponding to 6 slices of metabolite data, were used in the structure/metabolite analysis.

An average imaging session with the above-described protocol took about 1 hour.

IMAGE ANALYSIS

Spectroscopic Images

Six MRSI slices were used, beginning with the slice 12.8 mm above the anteroposterior commissure line and extending superiorly (Figure 1). These slices were chosen because they had the least amount of signal loss and artifacts due to field inhomogeneity. Within these 6 slices, only pixels with good homogeneity (B0 shifts within the range of ± 5 Hz) were included for analysis. These slices were also manually edited to remove regions, usually outside of the brain, of obvious lipid and/or water artifacts. To further guard against the possibility that incompletely suppressed water signal contaminated the MRSI data, especially for Cho and Cr concentrations in the medial frontal region, an exclusion region roughly corresponding to the cingulate gyrus was constructed for each slice by proportionate geometric positioning. The metabolite signals were calculated as magnitude values, so the noise in the metabolite maps had a non-Gaussian (Rician) noise distribution in the low signal-to-noise ratio range. To account for the effects of this non-Gaussian noise distribution, a bias correction was applied to the metabolite signal intensity values.29

METABOLITE CONCENTRATION AND TISSUE TYPE

Despite the significant tissue volume deficit in patients with AD, the number of spectroscopic voxels meeting criteria for analysis did not differ significantly among the 3 groups (F2,49 = 2.399, P = .10). The concentration estimation model provided separate metabolite concentration estimates for gray matter and white matter (Figure 3).

A repeated-measures analysis of variance (3 groups by 2 tissue types) for NAc concentration yielded significant effects of group (F2,47 = 6.913, P = .002) and tissue type (F2,47 = 234.108, P = .001) but no interaction (F2,47 = 1.708, P = .19). The overall group difference was significant in gray matter (F2,49 = 5.283, P = .008); the AD group had significantly lower gray matter NAc levels than the young (P = .03) and elderly (P = .009) control groups, which did not differ from each other. The ratio of gray to white matter NAc concentration was similar for the 3 groups (young control ratio = 1.31; elderly control ratio = 1.38; and patients with AD ratio = 1.30) (F2,49 = 1.024, P = .37).

For Cr concentration, a repeated-measures analysis of variance (3 groups by 2 tissue types) yielded significant group (F2,47 = 21.25, P = .001) and tissue-type effects (F1,47 = 884.214, P = .001) and interaction (F1,47 = 3.934, P = .03). For both tissue types, the elderly controls and patients with AD had higher Cr concentrations than the young controls (elderly vs young controls for gray matter: F12 = 4.678, P = .001, and for white matter: F12 = 3.935, P = .001; patients with AD vs young controls for gray matter: F12 = 4.619, P = .001, and for white matter: F12 = 2.572, P = .02). By contrast, the elderly con-

Structural Images

Nonbrain (ie, dura, skull, and scalp) tissue was removed. The images were converted into segmentation maps, with each pixel designated as gray matter, white matter, or cerebrospinal fluid, and volumes were derived for each compartment. The data from each of the 2 segmented, high-resolution, structural slices corresponding to a metabolite slice were combined to provide 128 (8×8×2) segmented voxels underlying each metabolite voxel. Brain tissue composition of each subject was computed, with and without the voxels excluded in the metabolite analysis (Figure 1).

STATISTICAL ANALYSIS

Group differences were tested with repeated-measures analysis of variance and Student t tests. We performed exploratory correlations between metabolite measures and cognitive test scores with Pearson product-moment correlations. We report correlations that reached P<.05 or less (1 tailed), with predictions that better performance would be associated with higher concentrations of NAc and lower concentrations of Cr and Cho; family-wise Bonferroni adjustment for 3 comparisons (3 tests for each metabolite) required P<.03. Correlations were done for the AD and elderly control groups independently to avoid the possibility that the results would merely reflect group differences.

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trol and AD groups did not differ significantly from each other in either gray matter ($t_{13} = 1.065, P = .29$) or white matter ($t_{13} = 1.386, P = .18$) Cr concentrations. Again, the ratio of gray matter to white matter Cr concentration was similar for the 3 groups (young control group ratio $= 1.98$; elderly control group ratio $= 1.95$; and AD group ratio $= 2.12$ ($F_{2,49} = 1.503, P = .23$).

For Cho concentrations, a repeated-measures analysis of variance (3 groups by 2 tissue types) yielded a significant group effect ($F_{1,47} = 10.964, P = .001$) and interaction ($F_{2,47} = 12.073, P = .001$) and trend toward a tissue-type effect ($F_{1,47} = 3.691, P = .06$). Gray matter Cho concentrations were lowest in the young controls and highest in the patients with AD, and all group-paired comparisons were significant (young vs elderly: $t_{13} = 3.925$, $P = .001$; elderly vs patients with AD: $t_{13} = 2.506$, $P = .02$; young vs patients with AD: $t_{13} = 4.993$, $P = .001$). As indicated by the interaction, white matter Cho showed a different pattern from gray matter; only the comparison between the elderly controls and patients with AD reached significance; the elderly group had higher Cho concentrations than the AD group ($t_{13} = 3.074$, $P = .004$). Unlike NAc and Cr, the ratio of gray matter to white matter for Cho concentration showed a group difference, with the value for the AD group higher (ratio = 1.20) than values for the young (ratio = .79, $t_{29} = 4.061$, $P = .001$) and elderly control (ratio = .91, $t_{13} = 3.362$, $P = .002$) groups; the young and elderly controls did not differ significantly from each other ($t_{13} = 1.351$, $P = .19$).

The 3 group-by-metabolite analyses of variance were recalculated excluding women, because the younger group was composed of men only. The results were the same without the women, except that the group-by-Cr interaction was no longer significant in the men-only analysis.

**CORRELATIONS BETWEEN METABOLITE CONCENTRATIONS AND COGNITIVE TEST SCORES**

As expected, the AD group was impaired on the 3 cognitive measures compared with the elderly controls (Table 3). There were several brain-behavior cor-

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Patients With Alzheimer Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong>*/Age, y/Sex MMSE† Medications</td>
</tr>
<tr>
<td>1/69/F 15 L-Carnitine, estrogen and acetaminophen</td>
</tr>
<tr>
<td>2/65/F 20 L-Carnitine, estrogen, and over-the-counter health food and vitamin supplements</td>
</tr>
<tr>
<td>3/76/F 21 Estrogen and aspirin</td>
</tr>
<tr>
<td>4/73/M 24 Lorazepam and over-the-counter vitamin supplements</td>
</tr>
<tr>
<td>5/79/F 15 Sertraline hydrochloride, hydrochlorothiazide, lisinopril, levothyroxine sodium, timolol optic, promethazine hydrochloride, and over-the-counter antihistamines</td>
</tr>
<tr>
<td>6/72/F 18 L-Carnitine, estrogen and acetaminophen</td>
</tr>
<tr>
<td>7/79/M 21 None</td>
</tr>
<tr>
<td>8/84/F 17 Furosemide, estrogen, mecizine hydrochloride, and metoprolol</td>
</tr>
<tr>
<td>9/73/M 20 L-Carnitine, estrogen and acetaminophen</td>
</tr>
<tr>
<td>10/73/M 24 None</td>
</tr>
<tr>
<td>11/77/F 26 Progesterone, estrogen, folic acid, melatonin, tacrine hydrochloride, hydrochlorothiazide, L-Carnitine, and over-the-counter health food</td>
</tr>
<tr>
<td>12/77/M 16 None</td>
</tr>
<tr>
<td>13/68/M 24 Estrogen and acetaminophen</td>
</tr>
<tr>
<td>14/78/F 11 L-Carnitine, hydrochlorothiazide, theophylline, and naphazoline hydrochloride</td>
</tr>
<tr>
<td>15/71/F 15 None</td>
</tr>
<tr>
<td>16/60/M 28 L-Carnitine</td>
</tr>
</tbody>
</table>

* Patient 3 had Crohn disease; patient 5 had depression; patient 8 had past depression and anxiety; and patient 16 had past alcoholism, with 18 years of sobriety.

† MMSE indicates Mini-Mental State Examination.

**Figure 1.** Left, Brain images of a 73-year-old male control. Right, Brain images of a 75-year-old male patient with Alzheimer disease. Superior to inferior brain slices are shown from left to right. Segmented image: the fast spin-echo magnetic resonance structural images are segmented into cerebrospinal fluid (dark gray), gray matter (medium gray), and white matter (light gray). Low-pass tissue image: The tissue image after low-pass filtering matches the spatial frequency characteristics of the metabolite images. The white outlines on these and the specific metabolite images below are taken from the perimeter of the structural images and indicate the registration of the metabolite image with the structural image. Images of N-acetyl compounds, creatine, and choline are of signal intensities that contribute to the quantification of each metabolite concentration.
relations in the predicted direction. In elderly controls, face recognition scores were positively correlated with gray matter NAc concentration ($r = 0.80, P = .001, n = 13$). In patients with AD, higher Cr gray matter concentrations were related to lower word-recognition scores ($r = -0.67, P = .03, n = 11$), and higher Cho gray matter concentrations were related to lower face recognition scores ($r = -0.70, P = .02, n = 11$).

Independent estimation of the concentrations of NAc, Cr, and Cho revealed different patterns across the groups: NAc showed a disease effect, Cr showed an age effect, and Cho showed disease and age effects. N-Acetyl compounds concentration was significantly reduced only in AD in gray matter but not white matter, even though both the AD and elderly control groups had substantial gray matter volume deficits relative to the young controls. Both the elderly healthy and AD groups had an excess of Cr in gray matter. Choline concentration in gray matter was notably higher in the elderly than in the young control group, and the AD values were even higher than those measured in the elderly controls.

While neuronal soma and processes may shrink, the current consensus is that little if any loss of neuronal cell numbers occurs in normal aging.16-20 Gray matter volume deficits without NAc concentration deficit suggests normal cell integrity in the healthy elderly. Like post-mortem studies,10,16-20,28 the current in vivo study revealed severe gray matter volume deficits and an accompanying deficit in gray matter NAc concentration in AD.

For literature comparison, we calculated whole brain raw signal intensity NAc/Cr ratio values of 1.382, 1.274, and 1.225 for the young, elderly, and AD groups, respectively. Concentration-corrected ratios of NAc/Cr for gray matter only presented a similar pattern of 1.38, 1.229, and 1.098 for the young, elderly, and AD groups, whereas for white matter only the pattern was 2.073, 1.734, 1.743. Thus, the NAc/Cr ratio differences seen in whole-brain mixed gray-white tissue represent a decrease in the NAc/Cr ratio with aging but no additional AD effect in white matter and a further decrease in this ratio in the AD group in gray matter.

Consistent with other in vivo31,44-47,70-72 and ex vivo or post-mortem13,34,73,74 reports, NAc concentration, whether expressed as a ratio of Cr or Cho or in absolute terms, was substantially lower in patients with AD than in age-matched controls. Kwo-On-Yeun et al34 also noted substantially more reduction in NAc in gray matter than in white matter in comparing patients with AD to controls. As in other studies, NAc signal intensity was greater in gray matter than white matter for all 3 groups in our study.

Across all 3 groups, the calculated Cr concentration in gray matter was almost twice that in white matter, whereas the Cho concentration was more uniformly distributed between gray and white matter. Given these age- and disease-related differences in metabolite con-

![Figure 2. Volumes, without metabolite criteria imposed, derived from the segmented magnetic resonance imaging structural data for each of the compartments. The 3 groups showed a stepwise increase in brain cerebrospinal fluid volume, where the young controls had the smallest volume and the patients with Alzheimer disease (AD) the largest volume; all pairwise differences were significant. Gray matter volume showed the complementary pattern of group differences. White matter volume, however, was deficient only in the AD group relative to the young and elderly control groups. Error bars indicate SEMs (young vs elderly $*$ $P = .06$). Please refer to Table 2 for t test results.](image)

### Table 2. Structural Composition (mL) of Brain Generating Magnetic Resonance Spectroscopy Signal (Mean ± SE)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Young Controls</th>
<th>Young Controls vs Elderly Controls</th>
<th>Elderly Controls</th>
<th>Elderly Controls vs Patients With AD</th>
<th>Patients With AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With Metabolite Criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>40.6</td>
<td>$t_o = 6.421, P &lt; .001$</td>
<td>71.3</td>
<td>$t_o = 4.562, P &lt; .001$</td>
<td>99.9</td>
</tr>
<tr>
<td>Gray matter</td>
<td>151.9</td>
<td>$t_o = 6.043, P &lt; .001$</td>
<td>106.9</td>
<td>$t_o = 3.756, P &lt; .001$</td>
<td>64.8</td>
</tr>
<tr>
<td>White matter</td>
<td>208.0</td>
<td>$t_o = .734, P = .47$</td>
<td>198.4</td>
<td>$t_o = 1.938, P &lt; .07$</td>
<td>173.8</td>
</tr>
<tr>
<td><strong>Without Metabolite Criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>69.8</td>
<td>$t_o = 8.206, P &lt; .001$</td>
<td>124.3</td>
<td>$t_o = 5.119, P &lt; .001$</td>
<td>168.7</td>
</tr>
<tr>
<td>Gray matter</td>
<td>231.6</td>
<td>$t_o = 11.52, P &lt; .001$</td>
<td>163.3</td>
<td>$t_o = 4.558, P &lt; .001$</td>
<td>133.2</td>
</tr>
<tr>
<td>White matter</td>
<td>261.2</td>
<td>$t_o = .294, P = .77$</td>
<td>257.9</td>
<td>$t_o = 1.966, P &lt; .06$</td>
<td>234.2</td>
</tr>
</tbody>
</table>

*AD indicates Alzheimer disease.*
centrations for gray and white matter, it is clear that neither Cr nor Cho concentration is constant, and, thus, they should not be used without regard to age and disease effects as referents in ratio expression of the NAc signal. The 3 group comparisons—young controls, elderly controls, and elderly patients with AD—help to disentangle disease effects from effects of normal aging. The former should be superimposed on the latter. Patients with AD did not significantly differ from the elderly controls in the volume of Cr in either gray or white matter, but both groups differed from the young controls. This pattern indicates that increased Cr concentration in patients with AD is attributable to advanced age rather than to the disease. The elevated gray matter Cho concentration in patients with AD, however, seems to be the result of the additive effects of advanced age plus disease. Chang et al noted decreased brain water content (noncerebrospinal fluid) with age, which could explain a relative decrease in cortical volume with the same number of cells, leading to increased metabolite concentration. Miller et al reported that increased choline reflects degree of cellular density in brain tumors. Adding gliosis to this aging effect could explain the additional increase in Cho level we observed in patients with AD. White matter Cho concentration showed the opposite pattern, ie, no increase with age but a decrease with AD. Moats et al observed age-related increases in Cho levels in normal elderly but no further differences between normal elderly and patients with AD. Our observed increased Cho concentration in gray matter may be the result of cell membrane turnover.

Pettegrew et al, using in vitro phosphorus 31 (31P) MRS, found elevated phosphomonoester levels in patients with AD. Brown et al showed that phosphomonoester, phosphomonoester/phosphodiesther, and inorganic phosphate levels were elevated in patients with AD compared with controls, whereas no significant differences in any 31P indexes were found by Bottomley et al. Similar findings were reported by Murphy et al. More recently, Gonzalez et al reported a 50% increase in phosphomonoester/phosphodiester levels in patients with AD, but unchanged B-nucleoside 5′-triphosphate, phospho-creatine, and inorganic phosphate levels in AD, suggesting that the phosphodiester difference reflected changes in the biophysical state of membrane phospholipids. Using quantitative 31P and 1H perchloric acid extracts, Klunk et al found increased myo-inositol, aspartate, L-glutamate, alanine, phosphocholine, and phosphodiester levels, and decreased phosphoethanolamine and NAc-L-aspartate levels. They concluded that compounds related to membrane degradation and excitatory neurotransmission increased in patients with AD, while those related to neuronal integrity and inhibitory neurotransmission decreased. Pettegrew et al

Table 3. Scores on Cognitive Tests

<table>
<thead>
<tr>
<th>Tests</th>
<th>Elderly Controls</th>
<th>Elderly Controls Patients With AD</th>
<th>Patients With AD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Boston Naming Test</td>
<td>38.3 (1.2)</td>
<td>28.3 (2.9)</td>
<td>28.3 (2.9)</td>
</tr>
<tr>
<td>Warrington Recognition Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Words</td>
<td>46.8 (1.1)</td>
<td>30.2 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Faces</td>
<td>42.3 (1.2)</td>
<td>31.3 (2.2)</td>
<td></td>
</tr>
</tbody>
</table>

*AD indicates Alzheimer disease.
†n = 11.
increased phosphomonoester and phosphocreatinine levels that preceded dementia for 1 subject.

The proton Cr signal is a combination of phosphocreatine and creatine.8 Thus, one cannot separate the contribution of creatine and phosphocreatine to the total Cr signal or relate findings directly to phosphorus spectroscopy studies, which, for instance, report a decreased ratio of phosphocreatine to inorganic phosphate in AD.9 Similarly, several Cho-containing compounds contribute to the Cho peak in proton magnetic resonance spectroscopy. As reviewed by Michaelis et al,22 Cho plasmogen (0.6 mmol/kg), glycerophosphorylcholine (0.4 mmol/kg), phosphorylcholine (0.4 mmol/kg), cytidine-diphosphate-choline (0.05 mmol/kg), acetylcholine (0.03 mmol/kg), and choline (0.02 mmol/kg) contribute to the "MRS choline signal. Contributions from 15- to 18-mmol/L lipid-soluble phosphatidylcholine are minor.22,23 In vivo 1P MRS could clearly play an important role in identifying the sources of the observed Cho as well as Cr signals in 1H MRSI. A limitation of our method is imposed by the inhomogeneity of the main magnetic field. While we applied second-order, nonlinear shims to improve the homogeneity, the remaining field inhomogeneities, particularly in the more inferior and frontal brain regions, limit the useful extent of the observed volume. Because we apply a late-echo acquisition, metabolites with short T2 relaxation times, such as myo-inositol, are not observed. The level of brain metabolite concentration seen with 1H MRSI appears to have functional significance, given the correlations found with cognitive measures. The metabolite concentration–memory test correlations are consistent with our previous work, which showed a selective relationship between these tests and degree of hippocampal volume loss in patients with AD.4,5 Together, these results lend support to the hypothesis that the prominent disease-related increase in gray matter Cho concentration marks cellular degeneration resulting in reduced NAc concentration and poorer cognitive function in AD.

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