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Magnetic resonance spectroscopy in AD

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Article abstract—Proton MR spectroscopy (MRS) studies have found both decreased *N*-acetylaspartate (NAA) and increased myo-inositol in the occipital, temporal, parietal, and frontal regions of patients with AD, even at the early stages of the disease. This diffuse NAA decline is independent of regional atrophy and probably reflects a decrease in neurocellular viability. Reports of such metabolite changes are now emerging in the mild cognitive impairment prodrome and in investigation of the medial temporal lobe. In vivo quantitation of neural choline in AD has been inconclusive because of poor test–retest repeatability. Less robust evidence using phosphorous MRS has shown significant phosphocreatine decline and increments in the cell membrane phosphomonoesters in the early, and possibly asymptomatic, stages of the disease. These phosphorous metabolite disturbances normalize with disease progression. Phosphodiester concentration has been found to correlate strongly with AD plaque counts. MRS of AD has therefore introduced new pathophysiologic speculations. Studies of automated MRS for AD diagnosis have reported high sensitivity and moderate specificity, but are yet to test prospective samples and should be extended to include at least two MRS regions of interest. MRS has promise for predicting cognitive status and monitoring pharmacologic efficacy, and can assess cortical and subcortical neurochemical change.

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Proton MR spectroscopy (^{1}H-MRS) is sensitive to within-individual changes in the concentration of brain metabolites over time on the order of 1 mmol/L,[1] permitting a volume of interest (VOI) of between 1 to 8 cm^3. Phosphorous MRS (^{31}P-MRS) can study the high-energy phosphate metabolites, yet is only about 5% as sensitive, and so requires a much larger VOI, between 15 and 40 cm^3. MRS thus presents a new opportunity for assessment of the biochemical composition of pathologic and healthy brain tissue in vivo. In this review we summarize and evaluate MRS studies of AD.

Technical issues. Careful selection of pulse sequence and echo time is required as each can affect the range of biochemicals measured in the MRS scan.[2] The particular method of quantitation is also important for interpretation of results: relative methods have used the physiologic water signal[3] or the cell creatine signal[4] as a reference, but this technique has the disadvantage of not definitively distinguishing between numerator or denominator meta-bolite changes. Absolute quantitation methods[5] are more accurate in this respect, but results from between labs have not been consistent[6] and clinical implementation of current techniques is impractical.

An alternative to consider may be a two-fold relative quantitation system. Concordant findings based on referencing of metabolite peaks to both the CSF-corrected water signal and the creatine signal are likely to reflect real biochemical change.[7] Discordant findings may require further investigation with absolute techniques.

What do the metabolite peaks signify? The major ^{1}H-MRS metabolites are described below (see figure):

1. *N*-acetylaspartate (chemical shift [δ] = 2.02 and 2.6 parts per million [ppm]). *N*-acetylaspartate (NAA) exists in the brain at an approximate concentration of 12 mmol/L and has been found elevated in Canavan's disease and decreased in areas of focal neurologic pathology. Given that NAA is predominately intraneuronal, it has been widely used as a marker of neuronal density.[8-10] Reliable in vivo assay of NAA is suggested by strong correlations between in vitro estimates and MRS estimates in rat models[11] and its excellent signal characteristics. Although the

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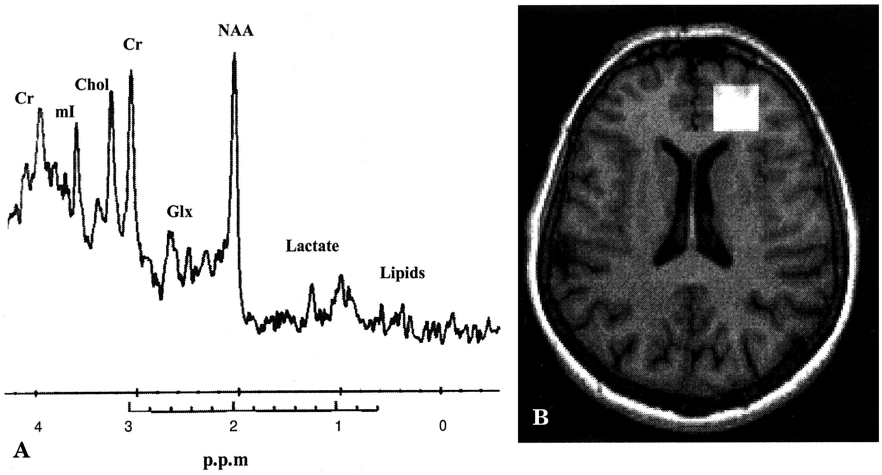

Figure. (A) Proton MR spectroscopy (^{1}H-MRS) spectra of frontal white matter (2 × 2 × 2 cm) in a 74-year-old woman. Acquisition was with the STEAM pulse sequence; repetition time, 1500 msec; echo time, 30 msec. Major metabolites are labeled: N*-acetylaspartate (NAA), free cholines (Cho), creatine plus phosphocreatine (Cr), and glutamate plus glutamine (Glx). Chemical shift is represented on the x-axis in parts per million (ppm). The NAA/Cr ratio in this example was 1.15. (B) Axial slice T1-weighted MRI shows the location of frontal lobe MRS voxel. T1-weighted MRI. Left side of figure = right side of brain.*

precise physiologic role remains uncertain,[10] a recent in vitro report suggested that NAA may reflect myelination processes in the adult human.[12] NAA has been found to undergo reversible changes in patients with relapsing MS,[13] on recovery from brain injury,[14] and in some patients with AIDS dementia complex after drug therapy.[15] A highly significant correlation has furthermore been found in vitro between mitochondrial phosphorylation and rates of NAA synthesis.[16] NAA may therefore be a useful in vivo marker of neurometabolic fitness, reflecting a level of neural viability that can recover after insult.

2. Choline compounds (δ = 3.2 ppm). Choline is a rate limiting precursor in the synthesis of acetylcholine and a precursor to cell membrane phosphatidylcholine. The choline MRS peak measures total levels of mobile choline, which include free choline, acetylcholine (present in relatively minute quantities), glycerophosphorylcholine (a byproduct of phosphatidylcholine breakdown), and phosphocholine (a phosphatidylcholine precursor). Membrane phosphatidylcholine is invisible on MRS. Correlations between regional choline levels of brain tumors and biopsy analysis show a significant association.[17] On the other hand, repeatability studies have found poor test–retest reliability.[1,18]

3. Myo-inositol (δ = 3.6 and 4.0 ppm). Myo-inositol (MI) is a largely mysterious sugar-alcohol whose structure is similar to that of glucose. It is estimated that 70% of the MI peak comes from free MI directly and 15% from MI phosphate.[19] MI may act as a marker of glial cell numbers, an osmoregulator, intracellular messenger, or detoxification agent in the brain as in the liver.[19]

4. Creatine plus phosphocreatine (Cr) (δ = 3.0 and 3.9 ppm). The phosphocreatine–creatine equilibrium reaction acts as a reserve for high energy phosphates and buffers cellular ATP/ADP ratios.[9] The combined "Cr" signal thus reflects the health of systemic energy use and storage. As mentioned, Cr has been used as a reference metabolite to quantify other neurochemicals. Repeatability studies have shown it to be stable over the course of months within an individual.[20] Factors known to affect Cr include age[21] and white matter disease.[22]

5. Glutamate–glutamine complex (δ = 2.1 to 2.4 ppm). The complex chemical structures of glutamate and glutamine mean that their peaks are hard to distinguish and are commonly labeled "Glx." Glutamate functions as the major excitatory neurotransmitter in the brain. Glutamine may be important to brain function, serving a role in detoxification and regulation of its precursor glutamate[19] within the astrocyte body.

Oxidative metabolism in the brain, as in muscle tissue, is notable for its high levels of phosphocreatine–creatine activity and a high steady state of mitochondrial respiration.[23] ^{31}P-MRS allows assessment of these different high-energy chemicals. The major peak assignments in ^{31}P-MRS include: 1) α, β, and γ nucleotide triphosphates, which reflect ATP levels; 2) phosphocreatine, a key indicator of oxidative metabolism (along with ATP); 3) phosphodiesters (PDE), comprised of resonances from glycerophosphorylcholine and other elements of the phospholipid bilayer[24] and therefore reflecting levels of cell membrane breakdown products; 4) phosphomonoesters (PME), mainly composed of signal from phosphocholine and other key precursors of membrane phospholipids; and 5) inorganic phosphate, used to estimate intracellular pH.[25]

The neurochemistry of Alzheimer's dementia. *Phosphorous MR spectroscopy.* Early work in this field involved the study of postmortem AD brain tissue. Pettegrew et al.[25-27] reported increases in PME and PDE, with one study finding a strong association between PDE and senile plaque counts.[27] A recent postmortem study[28] at high field strength reported that this abnormality may be greater in AD brains positive for the *APOE*-ε4 allele.

Clinical MRS studies have produced mixed results, with three studies[29-31] suggesting no differences in PME and PDE levels between patients with AD and control subjects. Results in reports from Pettegrew's laboratory, on the other hand, suggest a complex relationship in vivo.[32-34] PME concentration

Table *Main findings from MRS studies of AD*

MRS modality	Main AD findings	Comment
^{1}H-MRS	Decreased NAA ~15% throughout cortex in early disease and independent of MRI findings	High concordance
	Increased MI ~20% in white and grey matter in AD and MCI	High concordance
	No choline change in AD	Conflicting reports
	NAA correlated with plaque numbers	Few studies all agree
	MI correlated to neurofibrillary tangles	Few studies all agree
	NAA/MI ratio predicts cognitive decline	Few studies all agree
	NAA and MI alterations in vivo follow pathologic progression	New findings emerging
	Automated dementia diagnosis using NAA/MI ratio shows promising sensitivity and specificity	Need to replicate using >1 VOI and a prospective sample
	Possible to monitor neurobiological effects of drug treatment	New findings emerging
^{31}P-MRS	Decrease in PCr in early disease followed by normalization	Conflicting reports between laboratories
	Increase in PME in early disease followed by normalization	Conflicting reports between laboratories
	Increase in PDE correlates with disease severity and plaque counts	Few studies all agree
	PME, PCr, and PME changes precede any clinical symptoms	Single case report

NAA = *N*-acetylaspartate; MI = myo-inositol; MCI = mild cognitive impairment; PCr = phosphocreatine; PME = phosphomonoesters; PDE = phosphodiesters; MRS = MR spectroscopy; VOI = volume of interest.

has been found to increase in the mild stage of AD and then to fall to normal levels as the disease advances. The phosphorous metabolism marker phosphocreatine has been found to follow the converse pattern: it is depleted in mild AD and renormalizes in the later stages. On the other hand, neural membrane breakdown products, reflected by the PDE resonance, have been convincingly shown to fall with age in control subjects[33] and yet increase linearly with dementia progression.[32-34]

Interestingly, the same authors reported a normalization of phosphate metabolites after a year-long trial of acetyl-L-Carnitine, in conjunction with attenuation of symptom progression.[35] However, a 21-day trial of metrifonate (an acetylcholinesterase inhibitor), which improved clinical variables, failed to demonstrate phosphorous metabolite change.[36] Whether this was due to the medication not affecting phosphorous metabolism, or if such changes were masked by the low sensitivity and resolution of ^{31}P-MRS, is unresolved. A clinical finding of particular interest, and which requires follow-up investigation, is a case report of phosphorous metabolism changes preceding clinical symptoms altogether.[32] ^{31}P-MRS studies in AD are summarized in the table (a more comprehensive summary table appears in the online version of this article at www.neurology.org).

There are a number of limitations to these studies. Postmortem studies are dependent upon time until fixation and close attention to this variable has been lacking. The method of absolute quantitation used in the negative reports has the disadvantage of sampling particularly large brain regions and has lacked demonstrated repeatability. On the other hand, all of the positive ^{31}P-MRS findings have in effect come from a single laboratory, detracting from confident generalization.

Despite these limitations, the published reports suggest that in early, and possibly presymptomatic AD, a distinct biochemical abnormality is evident using regional ^{31}P-MRS. PME, the membrane lipid precursor group, becomes elevated and then falls back to normal concentration with dementia progression. Phosphocreatine levels move in the reverse direction, dropping off in mild dementia and recovering later. PDE, on the other hand, increase in step with senile plaque counts, a pattern quite distinct from the limited reports in the normal elderly of a gradual decline of this metabolite.

These conclusions warrant two important qualifications. First, MRS changes may not be as significant or extensive as the abnormalities in glucose metabolism observed in AD.[31] If, however, these biochemical changes can be detected early in the disease process, they may have major diagnostic implications. Second, few data indicate that these changes are specific to AD. Whereas one study[32] showed successful discrimination of AD from multiple subcortical infarct dementia, another study showed that an "other dementias" group could not be accurately distinguished.[37] Further metabolite information, particularly that resolved by ^{1}H-MRS, may be useful in this regard.

Proton MR spectroscopy. Proton MRS has yielded a growing body of interesting and largely replicable evidence of characteristic metabolite changes in AD, summarized in the table (a more comprehensive summary table appears in the online resource at www.neurology.org). A consistent finding has been a reduction in NAA levels in AD brains. This has been demonstrated in the occipital lobe,[20,38] temporoparietal region,[39] temporal lobe,[40-43] parietal lobe,[44] and in the frontal lobe.[45] Overall, NAA decrease in AD has been shown in at least 18 reports,

including in vitro studies showing a correlation with AD pathology.[37,46] Cortical NAA levels do not seem to be related to dementia severity and the decrement is indeed remarkable for its consistent magnitude—about 10% to 15%—across studies with different protocols and between different brain regions. Also, NAA depletion seems higher in gray matter compared with white matter. Studies that have attempted to control for CSF in the VOI suggest that the NAA depletion found in AD is independent of the level of atrophy.

Another striking finding in the literature has been the unforeseen elevation of MI levels by about 15% to 20% in the gray matter of patients with AD. Subjects with age-associated memory impairment show no significant increase in MI in the temporoparietal region,[39] yet one study demonstrated an increased MI signal in the posterior cingulate of individuals with mild cognitive impairment.[41] No significant MI changes have been confirmed in white matter but a moderate inverse association between frontal white matter MI levels and global mental function has been found.[40]

Whereas the clinical specificity of the NAA decline in AD is poor, the addition of MI information increases the accuracy of the diagnostic function. The combined NAA/MI ratio is robust in discriminating possible AD cases from age-matched control subjects.[18,40,47] For the more challenging task of discriminating AD cases from other possible dementia diagnoses, NAA/MI ratio was reported to yield a modest positive predictive value (74%). A negative MRS result in a subject with possible dementia was of more clinical utility; the same study estimated an 80% negative predictive value.[47] A smaller study using a 1.0-T magnet replicated the MI elevation seen in AD and found that this measure could be used to discriminate from patients with multi-infarct dementia.[38] Automated AD diagnosis using MRS seems eminently possible, but future studies will require validation of the discriminant function on a prospective sample. More than one VOI should be incorporated into any future studies testing MRS diagnostic ability.

The NAA/MI ratio in patients with AD has also been shown to significantly correlate with Mini-Mental State Examination (MMSE) scores,[18] and even to significantly predict MMSE change 12 months later.[43] Cognitive change has also been related to MI and NAA alteration in the hippocampi of individuals with AD.[42,48] Further research is necessary, but there are intriguing suggestions that ^{1}H-MRS may have a useful role in prognosis of mental function and tracking of disease progression.

A noteworthy finding has been the equivalence of in vivo choline estimates between pathologic groups and control subjects. Furthermore, although it is known that choline levels in the hippocampus of normal individuals are higher than when compared with other brain regions,[49] reports have so far failed to show a difference in hippocampal choline levels between patients with AD and control subjects.[42,48] Given that the choline peak seems to demonstrate higher variability when assayed with MRS methods,[18] strict correction for age, disease severity, medication status, and dietary intake is necessary to make accurate conclusions.

Pathophysiologic implications. *Membrane structure.* The phospholipid and high-energy phosphate pathways have together been implicated in the early and possibly preclinical stages of AD.[34] An explanation for early PME elevation may be blockage in the conversion of PME to membrane phospholipid. Abnormal phosphorylation of protein,[50] and the known kinase and protease activations that contribute to β-amyloid deposition,[51] may contribute directly to structural weakness in neurons via disruption of PME-phospholipid enzymes. Such structural vulnerability can make neurons more susceptible to lesion-induced toxicity and death. The biochemical consequence of this—decreased membrane phosphatidylcholine—has been found in AD brains when compared with controls.[25] Pharmaceutical interventions based on this rationale may be worth further investigation, given the ease of in vivo assay of PME.

As AD increases in severity, PME levels have been shown to normalize. This may reflect the influence of biochemical factors that link amyloid deposition and cell membrane synthesis; however, it is clear that a better understanding of this discontinuity is required.

PDE levels, reflecting cell membrane breakdown products, are highly correlated with senile plaque numbers ($r = 0.89$).[27] The progression of AD, with the increase in density and number of senile plaques, can therefore be quantified in vivo by the increase of the PDE groups. Researchers and clinicians appear to have hitherto ignored this important finding. Because in normal subjects PDE levels decline with age, possibly reflecting a loss of membranous vesicles, this relationship may aid the differential diagnosis of dementia when serial phosphorous spectroscopy is possible.

High-energy phosphate metabolism. Pettegrew et al.[32-34] showed phosphocreatine depression in the early clinical stages followed by normalization. Depletion of high-energy buffering groups not only impairs neural function but may also leave neurons vulnerable to oxidant toxicity, stress-induced apoptosis, DNA mutation, and other harmful effects. Given that the main calorific requirement in the neuron is for neurotransmitter release and cycling,[52] drug therapy aimed at reducing the neuroenergetic deficit early in the disease may be indicated and has the advantage of reliable in vivo measurement.

Metabolic disturbances in AD are well documented even after controlling for atrophy in the ROI.[31,53] It is therefore possible that phosphocreatine depletion may provide an additional preclinical marker. Cortical glucose metabolic rates decrease, however, with dementia progression while phosphocreatine levels normalize. Longitudinal studies with

these two markers, when combined with neuropsychiatric assessments, may yield useful data.

Neural viability. Methods based on absolute quantitative techniques in vivo and on postmortem analysis have concluded that an approximate 10% to 15% reduction in NAA concentration occurs in the gray matter of individuals with AD. This change appears to be independent of the degree of atrophy as seen on MRI. These findings have been inconsistently understood in terms of neuronal loss or decreased neural density.

NAA depletion may well represent two separable processes: actual neural cell death in the brain region under examination, and another process reflecting a decreased level of functionality or metabolic integrity in the neurons that are still viable. Abnormalities seen on MRS may anticipate anatomic degeneration discerned on MRI. Reports of the reversal of NAA deficits in certain neurologic conditions support the latter "cellular fitness" interpretation. The functional significance of the NAA variance is underlined by studies showing that NAA levels covary with cognitive performance[54]; one investigation using a healthy elderly sample demonstrated that this relationship is independent of other neurocognitive predictors and is specific in terms of topographic locale and neuropsychological domain.[7]

NAA variation in structurally sound tissue may therefore reflect processes related to altered mitochondrial activity[16] or neuroinflammation.[55] This is not inconsistent with the understanding of AD as an inflammatory reaction,[56] or investigation of the mitochondrial basis of neurologic disorders,[57] so that the quantitation of NAA may be of value when attempting to monitor pharmacologic efficacy. Whether NAA depletion in AD can be partially reversed by treatment, pharmacologic or cognitive–behavioral, is a question worth examining.

Reductions in NAA have been found to be widespread in the cortex and to a lesser degree in the white matter of individuals with AD, suggesting that not only are neural cell bodies damaged, but axonal injury may also be involved. The use of MRS to investigate the temporal primacy of cortical metabolic decline versus axonal decline in AD is of great interest. In contradistinction to the progression of structural changes in AD,[58] this pattern of neural peptide depletion, in addition to the early membrane changes seen with ^{31}P-MRS, indicates that a diffuse insult to neurocellular viability early in the disease process may be in effect.

Inositol disturbance. In studies using short echo times, MI levels were found elevated. Similar elevations have been found in the frontal lobes of patients with frontotemporal dementia[59] and the basal ganglia of those with Huntington's dementia.[60] Understanding the significance of the MI increase is difficult given the complexity of its biochemical pathways, a summary of which is provided by Ross et al.[61] A simple explanation is based upon the enzymatic conversion of MI to inositol triphosphate (IP_3).[62] IP_3 functions as an important intracellular second messenger that bonds to plasma membrane. Alterations in this compound can have various neurophysiologic consequences, such as impairment of cholinergic activity and inhibition of Ca^{2+} release. Consistent with this explanation is the finding that IP_n levels are depleted in the hippocampi of patients with AD.[63]

There are other possible explanations of MI alterations in AD. Inhibition of MI conversion to neural membrane phosphatidylinositol could lead to MI accumulation and phosphatidylinositol depletion, a result also found in postmortem AD brains.[64] MI may also act as a marker of neuropathologic lesions, a suggestion supported by the report of a strong correlation between the MI signal and neurofibrillary tangles.[37] Glial cells in general have a high MI content,[65] and there is related evidence that MI and choline levels increase in areas of high cellularity.[17]

Another line of speculation is that MI disturbance has its origins in the kidney rather than the brain. Chronic renal failure induces MI inflation and NAA depletion in a pattern similar to that found in AD.[47] One study using proton MRS found that patients undergoing hemodialysis and undialyzed patients with chronic renal failure had a 14% increase in neural MI levels.[66] Transplantation reversed these metabolic anomalies. The possible role of aluminum neurotoxicity in patients undergoing dialysis and those with AD[67] should also be considered, although the evidence remains inconclusive.

MI is known to break down into glucuronate, which plays a detoxifying role in the liver.[19] Patients with diabetes, carbon monoxide poisoning, and AD all demonstrate cerebral MI elevation,[47] and these entities may be thought to be related through neurotoxicity. In a wider sense, a decline in detoxification efficacy has been implicated in aging and dementia.[68] Although the hypothesis is untested, MI may play a similar detoxification role in the brain, and its elevation in AD may reflect increased neurotoxic load rather than pathology per se.

Although the meaning of MI elevation remains unclear, it seems that such a finding may have a prognostic value as well. Investigations of Down's syndrome suggest that MI alteration may precede and predict subsequent cognitive decline.[69] Similar associations between MI levels and changes in MMSE scores have been reported in AD.[40,43]

Cholinergic imbalance. Perhaps the most perplexing finding to come from ^{1}H-MRS study of AD has been the lack of a detectable abnormality in choline levels. Loss of cholinergic neurons in AD, particularly in the hippocampus, predicts an increase in soluble free choline and glycerophosphorylcholine. In vitro studies have confirmed higher free choline and glycerophosphorylcholine levels in AD brains.[61] Of the 15 in vivo MRS studies reviewed, however, only two reported a significant choline elevation in AD.[70,71] Interestingly, both studies used chemical shift imaging (CSI) methods, and one used increased

magnet strength (2 T), which can increase the sensitivity of signal detection. Evaluation of these findings is difficult, however, given CSI interpretation is still developing. Single-voxel MRS in regions of AD cholinergic alteration as prescribed by CSI may help resolve these contradictions. The poor repeatability of choline quantitation[17] may otherwise be due to the rapid metabolism of dietary choline.[9]

Measurement of intraindividual change may be another way to obtain more accurate choline information. One group examined the effect of xanomeline, a muscarinic agonist, in the midparietal lobe of subjects with AD at baseline and 2 months after drug therapy.[72] The prediction was that choline therapy should promote decreased cholinergic membrane breakdown and so lead to relative decreases in MRS-visible choline. Choline was found significantly decreased in the drug takers versus placebo control subjects. An interesting follow-up report found a greater choline decrement in pharmacologic responders versus nonresponders.[73] The combination of therapeutic intervention, repeat spectroscopy, and cognitive assessment is likely to be a profitable and interesting research design in the investigation of AD.

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