Longitudinal Changes During Aging Using Proton Magnetic Resonance Spectroscopy

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Objective. We aimed to examine the longitudinal change in proton magnetic resonance spectroscopy (1H-MRS) visible metabolites (N-acetyl aspartate [NAA], creatine [Cr], choline [Cho], and myo-Inositol [mI]) in brains of elderly individuals over 3 years and relate them to cognitive function.

Methods. Neurologically and psychiatrically normal volunteers (n = 40) were examined at baseline and 3 years later with 1H-MRS in two voxels (frontal white matter n = 29, and occipitoparietal gray matter n = 36) and with detailed neuropsychological assessments. Longitudinal analyses were performed with age, educational level, sex, and white matter hyperintensities (WMH) in voxels as covariates.

Results. Frontal mI was significantly increased over time in male participants, but all other metabolites were stable over time. Neuropsychological performance was not significantly changed over 3 years, and there was no relationship between change in metabolite levels and change in neuropsychological function.

Conclusions. MRS-visible metabolites are stable in elderly persons over 3 years, with the exception of mI which shows an increase. Increasing mI may be a marker of aging or a preclinical neurodegenerative process. MRS changes do not correlate with change in neurocognitive function during aging.

LONGITUDINAL neuroimaging studies of aging have mainly been performed using structural magnetic resonance imaging (MRI). These studies have found reduced gray matter density over 3 years in the prefrontal, medial temporal, and the posterior parietal regions (1–3). Total brain white matter hyperintensity (WMH) increases were small over 2 years (4), with larger changes observed over 4 years, particularly in the left hemisphere, suggesting changes in myelination (5). Regional effects of white matter changes were more apparent in frontal and temporal regions, but not in the occipital and parietal regions (5).

Metabolite changes with age have been investigated in many studies using proton magnetic resonance spectroscopy (1H-MRS) but with variable results. These studies measure a number of compounds in the brain, most commonly N-acetyl containing compounds (NAA), creatine and phosphocreatine (Cr), choline-containing compounds (Cho), and myo-Inositol (mI). NAA is thought to be a marker of neuronal viability and metabolic efficiency because its production rate parallels oxygen consumption and adenosine triphosphate (ATP) production in the mitochondria (6). Changes in Cho indicate changes in membrane synthesis, and can often indicate gliosis, myelination, or inflammation. Cr is involved in energy metabolism, and mI is a putative glial cell marker that is involved in second messenger functioning, and is found to be increased in age-related disorders, such as Alzheimer’s disease (AD) (7). The measurement of these metabolites is important as it provides additional information about brain function to structural MRI as changes in metabolites can occur in tissue without abnormalities on structural MRI, and metabolite changes have been shown to be associated with cognitive changes (8). Additionally, a number of 1H-MRS studies have observed age-associated changes in metabolites.

Most 1H-MRS studies of aging are cross-sectional, thereby complicating the interpretation of age-related changes. Studies that include participants ranging from young adulthood to senium have observed reduced NAA (9,10) and increased Cr, Cho (11–15), and mI (12) in various regions of the brain as it ages. In cross-sectional studies of elderly participants only, the reported correlations between metabolites and age are more variable, with an increase in Cho and Cr and an increase, decrease, or no change in NAA (16,17). Many factors may contribute to the discrepancies between studies, including the different quantitation methods and acquisition protocols, changes in the tissue composition within the voxel, differential regional and gender effects, and correction factors.

The dynamic changes in metabolites with age are best examined in longitudinal studies. Reproducibility of 1H-MRS has been demonstrated in several studies (12,18–21), allowing researchers to follow-up participants over time with reasonable accuracy. The repeated examination of the brain with 1H-MRS using the same scanner and imaging protocol can address some the concerns relating to varying methodologies. The study of a wide age range in the elderly group is important as the changes may not have a linear relationship with age.

Gender differences are also important in aging studies, because gender effects appear to emerge with increasing age. NAA/Cho may be higher in men in the right centrum semiovale only at older ages (22). In a large 1H-MRS imaging
study, men had lower Cho, Cr, and NAA in the frontal white matter and in the cingulate compared to women (16).

Longitudinal neuropsychological changes in aging indicate that there may be a selective decline in memory, with other cognitive domains relatively spared over 3 years (23). The longitudinal relationship between neuroimaging variables and cognitive change was examined in a study over 2 years, which observed small increases in WMH, but no association with the minimal changes in neuropsychological function that were observed over this period (4). No study has examined the relationship between longitudinal metabolite change and cognitive change.

Using 1H-MRS, we studied a group of cognitively normal elderly persons in an attempt to determine the longitudinal change over a period of 3 years in brain metabolites and the relationship between change in metabolites and change in cognition. The relationship between longitudinal metabolite change and changes in cognitive function must be examined to determine the significance of the MRS findings. It was hypothesized that metabolites would be stable in participants with no cognitive decline, but in persons with greater cognitive decline changes in metabolites would be more pronounced in the frontal lobe, in line with the frontal lobe hypothesis of aging (age-related brain changes are thought to be more pronounced in this region; 24). The occipitoparietal voxel is relatively easy to image and less likely to exhibit age-associated changes; therefore, it was chosen as a control region. Moreover, any MRS study must use multiple brain regions as the effects of aging are not uniform throughout the brain. To our knowledge, this study has the longest interval to test changes over time of 1H-MRS measures and cognitive performance in a sample of cognitively normal elderly participants.

**METHODS**

**Participants**

The study sample, comprising volunteers recruited from community organizations, was screened for absence of stroke, cognitive impairment, and psychiatric disorder by history and examination. These individuals were recruited as control participants for the Sydney Stroke Study (25). Exclusion criteria included neurological disease known to affect cognition; medical disease that was judged to affect cognition secondarily, including previous stroke or Transient Ischemic Attack (TIA), dementia, Mild Cognitive Impairment (MCI), Parkinson’s disease, multiple sclerosis, brain tumors, epilepsy; history of moderate to severe head injury; history of meningitis; report by informant of decline in the previous 5 years based on the IQCODE (26); mental retardation (Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition [DSM-IV] diagnosis); alcohol dependence; and contraindications to MRI (pacemaker, metallic foreign bodies, severe claustrophobia). A diagnosis of cognitive status was made by consensus, based on the medical, neurological, psychiatric, and neuropsychological assessments at a conference attended by two psychiatrists, a neurologist, and two or more research psychologists. We divided the neuropsychological test performance into cognitive domains, and a participant was considered to have impairment in that domain if the performance on a test within the domain was <5th percentile based on age-corrected norms, or to have marginal impairment if the performance was between the 5th and 10th percentiles. Definite impairment was necessary in at least two domains for dementia to be considered, for which functional impairment was also required. For MCI, definitive impairment in one domain was sufficient. This method of classification has previously been published by our group (25).

A flow chart shows the participants included at each time-point (Figure 1). Of the 112 control individuals recruited and enrolled, 86 agreed to have 1H-MRS. Due to an early change in the acquisition protocol and a change in the scanner, only 62 of these control individuals had useable 1H-MRS data in either voxel. Two individuals were excluded after scanning, because their neuropsychological data at baseline showed them to be cognitively impaired. Of the remaining 60 individuals, data were acquired for 47 in the frontal voxel and for 57 in the occipitoparietal voxel. These 60 participants have frontal voxel data, occipitoparietal voxel data, or both. The number of participants for whom useable frontal voxel data were acquired is lower because this area is harder to image due to proximity to the sinuses, resulting in larger line widths on average than those of the occipitoparietal voxel. Also, the greater chance of lipid interference in this region meant that some of the spectra were not able to be analyzed. The occipitoparietal region is easier to image; therefore, most of the participants have good quality data from this region. Movement during the imaging also reduced the number of scans that could be analyzed.

Of the participants with baseline data, repeat 1H-MRS was performed on average 2.50 ± 0.27 years later in 40 participants, with baseline and 3-year data available for 30 participants in the frontal voxel and for 36 participants in the occipitoparietal voxel. Poor quality data (line width > 10 Hz) or lipid interference left a sample of 30 participants (15 males, 15 females) for the frontal voxel and 36 participants (18 males, 18 females) for the occipitoparietal voxel with both baseline and 3-year data. Neuropsychological data were obtained for each participant at baseline, 12 months, and 3 years. The 12-month data are not presented here. Neuropsychological testing at baseline and 3 years was completed in 35 of the participants in this study. At follow-up, six individuals had withdrawn from the study, five refused a second scan, and nine had follow-up data that were not comparable, due to the replacement of the scanner before all the data had been acquired. There were no significant differences for age (t = 0.570, p = .570), sex (χ² = 0.233, p = .629), educational level (t = 0.734, p = .465), or Mini-Mental State Examination scores (t = 1.269, p = .207) between participants with and without follow-up. The power to detect a moderate effect size over 3 years was 81%.

**Assessments**

Neuropsychological assessment.—The neuropsychological battery comprised the following tests pertaining to various cognitive domains: attention {Digit Span Forward
(Wechsler Adult Intelligence Scale-Revised [WAIS-R]; 27), Mental Control (Wechsler Memory Scale, Revised [WMS-R]; 28); visual memory [Visual Reproduction I & II from WMS-R (28)]; verbal memory [Logical Memory I & II from the WMS-R; (28)]; mental flexibility [Color-Form Sorting Test (29) and Trail Making Test Part B (30)]; verbal fluency [Controlled Oral Word Association Test (31) and Animal Naming (32)]; abstraction [Similarities (WAIS-R; 27)]; working memory [Digit Span Backward, Arithmetic from WAIS-R (27)]; language [15-item Boston Naming Test (33)]; praxis–gnosis [Western Aphasia Battery, ideomotor apraxia subtest items (34), finger gnosis and stereognosis (35)]; visuospatial function [Block Design from WAIS-R (27) and copying simple figures]; and speed of processing [Trail Making Test Part A (30) and Symbol Digit Modalities Test (36)]. Mental flexibility and verbal fluency were together characterized as executive function.

Trained psychologists performed assessments. Participants were given breaks when appropriate to minimize the effects of fatigue on performance. Participants judged to be clinically depressed were not tested until their depression had been satisfactorily treated as shown by a total score on the Geriatric Depression Scale (GDS) of less than 5, a reduction in self-reported symptoms of depression, informant report, or further psychiatric assessment.

MRI/1H-MRS protocol.—MRI and 1H-MRS were performed using a 1.5 Tesla Signa scanner (GE Medical Systems, Milwaukee, WI), and included a scout midsagittal cut for AC-PC plane alignment (two-dimensional, repetition time [TR] 300 ms, echo time [TE] 14 ms; thickness 5 mm, number of excitations 1.5); a 1.5 mm-thick T1-weighted contiguous coronal sections through whole brain using a Fast Spoiled Gradient Recall (FSPGR) sequence and three-dimensional acquisition (TR 14.3 ms, TE 5.4 ms); and 4 mm-thick T2-weighted fluid-attenuated inversion recovery (FLAIR) coronal slices through the whole brain (TR 8900, TE 145, inversion time 2200, field of view 25, 256 × 192). 1H-MRS was performed in the left frontal region, consisting of mainly white matter (single voxel 2 × 2 × 2 cm³) and the occipitoparietal region, consisting of mainly gray matter (single voxel 2 × 2.7 × 2 cm³). The voxels were localized using the axial plane, with the frontal voxel positioned anterior to the frontal horn of the left lateral ventricle (maximizing the amount of white matter contained in the voxel) and the occipitoparietal voxel in the posterior midline (maximizing the amount of gray matter in the voxel) (Figure 2). The frontal voxel was chosen to include white matter tracts from the frontal-subcortical circuits, and the occipitoparietal voxel acted as a control representative gray matter region, which is relatively easy to image. Voxel placement at 3 years was carefully located to the same area using the T1-weighted images from the baseline scan. Automated shimming and water suppression were performed before acquisition using the STimulated Echo Acquisition Mode (STEAM) sequence with a 30-ms TE, 1500-ms TR, and 13.7-ms mixing time. Separate acquisition of the unsuppressed water signal was also performed. The number of data acquisitions was 256, averaged across 2048 data points.
Data Analysis

Neuropsychological tests.—Using the control group index assessment mean and standard deviation, z scores were derived. To determine the individual’s performance on a test, a z score was computed. A composite z score was then obtained by adding the z scores for all tests assigned to each cognitive domain divided by the number of tests contributing to that cognitive domain. A combined total z score for all tests was also calculated. The scores from the domain scores were used in the exploratory analyses.

\(^{1}\)H-MRS analysis.—The spectra from the \(^{1}\)H-MRS on the GE magnet were analyzed using MRUI-99x software (37) on MATLAB 5.3. The residual water peak was removed using time–domain Hankel–Lanczos singular value decomposition filtering. Time–domain fitting was then carried out for NAA, Cr, Cho, ml, lipid, and lactate peaks using Advanced Method for Accurate, Robust and Efficient Spectral (AMARES) fitting to measure the area under the curve, with input of prior knowledge. Specifically, the phase of all peaks was zero relative to the estimated zero-order phase, and the line widths of NAA, Cr, Cho, and ml were assumed to be equal. The area of the water peak was determined separately from the unsuppressed water scan using singular value decomposition filtering. Noise was taken from the last 100 data points. Typical water line widths for the frontal voxel were 4–10 Hz and from the occipitoparietal voxel were 2.5–6 Hz (Figure 3). Spectra with water line widths greater than 10 Hz were excluded from analysis. The line width was the full width of the peak at half height.

To correct for partial volume effects within the voxels, the proportion of cerebrospinal fluid (CSF) was estimated from the structural MRI data using ANALYZE software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). A semiautomatic segmentation algorithm developed in house was used (Dr. Wei Wen, Neuropsychiatric Institute, Prince of Wales Hospital, Sydney), with manual input of the threshold for brain tissue, determined individually from T1-weighted structural images. In this program, pixels from within the voxel with an intensity level greater than the level determined by the rater were defined as brain tissue, and those below were defined as CSF. Isolated pixels below the threshold did not contribute to the proportion of brain tissue. To provide the corrected water value, \((1 - \text{the proportion of CSF})\) was multiplied by the unsuppressed water value for each voxel, and then values for NAA, Cr, Cho, and ml were calculated assuming a water concentration of 45 M. The proportion of WMH was estimated for each voxel from the FLAIR images by using a semiautomatic segmentation algorithm, with manual input of a threshold value to differentiate between normal tissue and WMH. The intra-rater reliability using intraclass correlation coefficients for five randomly selected control participants was \(r = 0.962\) in the frontal voxel \((p < .004)\) and \(r = 1.000\) in the occipital voxel \((p < .0001)\).

Figure 2. Localization of voxels in the axial plane for (A) the frontal white and (B) the occipitoparietal gray matter.

Figure 3. A representative spectrum from the occipitoparietal voxel.
LONGITUDINAL $^1$H-MRS CHANGES WITH AGE

Table 1. Change in Neuropsychological Performance From Baseline to 3 Years

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Composite $z$ Scores Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>MMSE/30</td>
<td>28.9 (1.49)</td>
</tr>
<tr>
<td>Total $z$ score</td>
<td>0.85 (8.58)</td>
</tr>
<tr>
<td>Attention</td>
<td>0.00 (0.72)</td>
</tr>
<tr>
<td>Visual memory</td>
<td>-0.24 (0.85)</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>0.06 (0.97)</td>
</tr>
<tr>
<td>Executive function</td>
<td>0.07 (0.81)</td>
</tr>
<tr>
<td>Abstraction</td>
<td>0.30 (0.83)</td>
</tr>
<tr>
<td>Working memory</td>
<td>0.05 (0.81)</td>
</tr>
<tr>
<td>Language</td>
<td>0.07 (0.84)</td>
</tr>
<tr>
<td>Praxis-gnosis</td>
<td>-0.07 (2.01)</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>-0.03 (0.36)</td>
</tr>
<tr>
<td>Speed of processing</td>
<td>0.09 (0.65)</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; MMSE = Mini-Mental State Examination.

Reproducibility analysis.—Reproducibility analysis was performed in five participants for the frontal voxel, and in six participants for the occipitoparietal voxel. These individuals were scanned twice on the same day. Participants were removed from the sample between scans, and voxel placement was carefully located to the same area. In the frontal voxel, the coefficients of variance (CVs) were 12.0% for NAA/H$_2$O, 13.9% for Cr/H$_2$O, and 24.9% for mI/H$_2$O, with no significant differences between the first and second measurement. In the occipitoparietal voxel the CVs were 7.1% for NAA/H$_2$O, 3.9% for Cr/H$_2$O, 8.3% for Cho/H$_2$O, and 27.5% for mI/H$_2$O, with no significant differences between the first and second measurement.

Statistical Analysis

Univariate repeated-measures analysis of covariance was used to assess the longitudinal change in neuropsychological and neuroimaging variables. For the neuropsychological data, age and educational level were treated as covariates. For the $^1$H-MRS data, the effects of age, sex, and atrophy were examined using these variables as covariates. In the frontal voxel, the effect of the presence of WMH on the metabolites was tested using the change in WMH within the voxel over 3 years as a covariate. The relationship between the change in metabolites and age was examined using Pearson correlation coefficients, with the data stratified by age at baseline into elderly (58–70 years) and older elderly (71–84 years). To assess the relationship between cognitive change and metabolite change, the change in cognitive domain scores and total $z$ score was correlated with the change in metabolites over 3 years.

RESULTS

Neuropsychological Changes Over 3 Years

There was a significant increase in Mini-Mental State Examination scores over time (baseline score = 28.9; 3 year score = 29.3, $t = 6.891$, $df = 1.34$, $p = .013$). This increase was possible due to practice effects, as there were no significant changes over time in activities of daily living (ADL) ($t = 1.41$, $p = .170$) and instrumental activities of daily living (IADL) ($t = 1.34$, $p = .326$). Visuospatial function was significantly improved ($F = 10.592$, $df = 1.34$, $p = .003$), but no other cognitive domain was significantly changed over time (Table 1).

$^1$H-MRS Changes Over 3 Years

In the frontal voxel, there was an overall significant multivariate effect for time, controlling for age, frontal voxel WMH, and atrophy ($F = 3.721$, $df = 4.21$, $p = .018$). Post hoc tests found that mI was significantly increased over time ($F = 6.474$, $df = 1.29$, $p = .018$) (Table 1). There was a significant interaction between sex and time indicating that the increase in mI was only occurring in the males ($F = 7.014$, $df = 1.29$, $p = .014$). There were no significant effects for sex, age, atrophy, and change in WMH and no significant interactions between these measures. In the occipital-parietal voxel, there were no significant effects for change in metabolites with time, with no effects for sex, age, or atrophy and no significant interactions between variables (Table 2). Similar, but less robust, results were observed for both voxels when these covariates were not included (data not shown).

Change in frontal NAA was significantly correlated with age at baseline in older elderly participants (>70 years) ($r = 0.500$, $p = .049$), but not in elderly participants (<70 years). Changes in occipitoparietal metabolites were not significantly associated with age.

Structural MRI Changes

There was no significant increase in the percentage of CSF within the frontal voxel over time. An increase in the percentage of CSF was observed in the occipitoparietal voxel over time, but this was not significant ($p > .05$). The proportion of WMH within the frontal voxel increased over 3 years ($F = 5.697$, $p = .025$). There were no WMH within the occipitoparietal voxel.

Changes in Metabolites and Cognition

Changes in total $z$ score and cognitive domain scores over 3 years were not significantly associated with change in any

Table 2. Neuroimaging Variables at Baseline and 3 Years

<table>
<thead>
<tr>
<th>Voxel Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 Year</td>
</tr>
<tr>
<td>Frontal</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>NAA</td>
<td>10.3 (1.2)</td>
<td>10.4 (1.0)</td>
</tr>
<tr>
<td>Cr</td>
<td>7.9 (0.9)</td>
<td>8.4 (1.0)</td>
</tr>
<tr>
<td>Cho</td>
<td>2.2 (0.4)</td>
<td>2.3 (0.3)</td>
</tr>
<tr>
<td>mI</td>
<td>3.3 (1.0)</td>
<td>3.8 (1.1)</td>
</tr>
<tr>
<td>CSF%</td>
<td>3.2 (3.1)</td>
<td>3.1 (2.7)</td>
</tr>
<tr>
<td>WMH%</td>
<td>0.8 (1.2)</td>
<td>1.2 (1.3)</td>
</tr>
<tr>
<td>Occipitoparietal</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>NAA</td>
<td>11.0 (0.7)</td>
<td>12.0 (1.3)</td>
</tr>
<tr>
<td>Cr</td>
<td>8.7 (0.7)</td>
<td>9.0 (0.9)</td>
</tr>
<tr>
<td>Cho</td>
<td>1.7 (0.3)</td>
<td>1.8 (0.3)</td>
</tr>
<tr>
<td>mI</td>
<td>3.8 (0.5)</td>
<td>3.7 (0.8)</td>
</tr>
<tr>
<td>CSF%</td>
<td>10.6 (7.0)</td>
<td>12.2 (6.2)</td>
</tr>
</tbody>
</table>

Notes: Values are mean (SD).

*aUnivariate statistic for change in metabolites from baseline to 3 years.

SD = standard deviation; N = number of participants; NAA = N-acetyl aspartate; Cr = creatine; Cho = choline; mI = myo-Inositol.
metabolite \((p > .05)\). The group was split in half by baseline neuropsychological performance, and correlations between change in metabolites and cognition were not observed in the lower performing or better performing groups. The changes in metabolites were not correlated with cognitive performance at 3 years.

**DISCUSSION**

This study observed relatively stable metabolite values over a period of 3 years, with the exception of an increase in frontal mI over time in elderly males. Cognitive function was stable or improved over time, with no relationship observed between changes in metabolites and cognitive function over time. The absolute metabolite values measured in the present study are in line with those from other quantitative studies \((38,39)\).

In the frontal white matter there was a significant increase in mI over 3 years with no significant changes in the occipitoparietal gray matter. Other longitudinal 1H-MRS studies have been performed over a time period of up to 1 year. In a study including adults age 22–62 years, there was no change in metabolites over 1 year in the parietal white matter \((14)\). In elderly persons, quantitative values and ratios were stable over a mean of 170 days and 260 days between scans in the parietal gray matter, with the exception of Cho, which was significantly decreased over 170 days \((39)\). In the hippocampus of 14 elderly persons, there were no significant changes observed in metabolites over 1 year \((40)\). These studies suggest that metabolites are relatively stable over time, in agreement with the current study, which showed stability over 3 years for NAA, Cho, and Cr. None of these studies observed a similar increase in mI over time; however, these studies were of shorter duration.

mI is a simple sugar-alcohol that is a putative glial cell marker \((41)\). It is a precursor for inositol lipid synthesis, and a constituent of membrane lipids \((41)\). It also acts as an organic osmolyte and is involved in second messenger system functioning \((42)\). An increase in mI has been observed in AD in many studies \([for a review see (7)], and mI/Cr is possibly an early marker for MCI and AD \((43)\). However, our study was very rigorous in determining neuropsychological state as well as medical and psychiatric data. Therefore, it is unlikely that MCI and AD patients were included in the study. It may be that participants had asymptomatic or presymptomatic AD pathology, such as that observed in postmortem studies \((44)\), that may explain the increase in mI over time. An increase in mI was observed prior to dementia with Down Syndrome \((45)\). It is impossible to test this without more follow-up of these patients.

The stability of NAA, Cr, and Cho in these participants over 3 years is not surprising, because cognitive performance was improved or stable over time. Previous research has observed a selective decline in memory function over 3 years in elderly participants, which was not observed in this study \((23)\). Practice effects are likely to be responsible for improvements in cognitive function, particularly because these participants also had neuropsychological testing at a 12-month assessment. Selective attrition and the demanding nature of the study may mean that there is selection of healthier elderly adults who are not necessarily representative of the general population.

A significant sex effect was observed for mI over time, with mI increased in males, but not females. Previous cross-sectional studies have found no significant gender differences in young to middle-aged adults in numerous brain regions including the frontal, temporal, thalamus, basal ganglia, and hippocampus \((14,17,46–49)\). Gender differences become more evident in studies of elderly persons. In a large study, men had lower Cho, Cr, and NAA in the frontal white matter and in the cingulate gyrus compared to women \((16)\). This finding is consistent with Positron Emission Tomography (PET) findings showing decreased glucose metabolism in the same region \((50)\); however, partial voluming was not corrected for in this study \((16)\), therefore the differences may be due to increased atrophy in males in these regions \((51)\). Decreased NAA/Cho in the right sided white matter and gray matter in the centrum semiovale was greater in males than females \((22)\), and higher NAA/Cr was observed in males in the left frontal white matter, but not after correcting for age \((52)\). Changes in metabolites may be complicated in aging for women by estrogen effects, with hormone replacement therapy nonusers showing an increase in Cho in the hippocampus and the parietal lobe with age \((53)\). In this study, we did not screen for hormone replacement therapy use; therefore, we could not test this.

There are a number of limitations to this study. First, we did not segment gray matter and white matter within our voxels. Although the voxels comprised primarily white matter or gray matter, this was not exclusively the case. We controlled for the presence of CSF using tissue segmentation by correcting the metabolite values for the percentage of tissue and CSF within the voxel. However, the inclusion of varying degrees of gray matter in the frontal voxel may affect the results. If gray matter were declining at a faster rate than white matter in this population, then the voxel at 3 years would contain less gray matter. This would mean that tissue changes may mask any real changes in metabolites, for example, the concentration of NAA is greater in the gray matter \((12,47,49)\).

Voxel placement is also an important source of error in repeat 1H-MRS studies. To minimize this, the voxels were carefully localized to the same place. Rose and colleagues \((39)\) showed that moving the voxel 5 mm in all directions from its initial position resulted in a maximum change in the gray matter volume to voxel ratio by only 6.5% in normal aging control participants. White matter changed maximally by 2.2% and CSF by 4.5%. We also performed a reproducibility analysis that indicated that the measurement error from voxel placement was unlikely to be a significant factor. Other studies in younger samples using similar acquisition protocols have observed CVs similar to those observed in the present study \((20,54,55)\). We observed similar CVs in the occipitoparietal gray matter for NAA, Cr, and Cho, but the CV for mI was greater than that in these previous studies. This finding may result from poorer water suppression in our study, due to the proximity of mI to the water peak. Similarly, the better reproducibility of Cr than NAA within our sample may be due to interference from the lipid and lactate peaks, which are more apparent in
older brains. The reproducibility measures were performed within 24 hours and are not necessarily representative of measures over 3 years. Therefore, it is possible that magnet instability affected the results.

Summary
We observed relative stability of cognitive function and metabolites in the frontal white matter and the occipitoparietal gray matter over 3 years, with the exception of an increase in ml in males. Changes in metabolites over time were not associated with changes in cognitive function. Further studies measuring a greater number of regions are required to fully characterize the whole brain changes occurring with age.

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