Biochemical Alterations in Multiple Sclerosis Lesions and Normal-appearing White Matter Detected by In Vivo $^{31}$P and $^1$H Spectroscopic Imaging

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The goals of the current study were threefold: first, to confirm previous single volume proton ($^1$H) magnetic resonance spectroscopy results of reduced N-acetyl aspartate (NAA, a putative marker of neurons) in multiple sclerosis (MS) white matter lesions using multiple volume $^1$H magnetic resonance spectroscopic imaging (MRSI); second, to measure the phospholipid metabolites phosphomonoesters and phosphodiesters in such lesions using phosphorus ($^{31}$P) MRSI; and third, to test the hypothesis that biochemical changes occur in the normal-appearing (on spin echo T2-weighted magnetic resonance images) white matter in patients with MS. Thirteen subjects with clinically definite MS were studied with both $^1$H and $^{31}$P MRSI, and 19 controls were studied with either $^1$H MRSI, $^{31}$P MRSI, or both. MS lesion, MS normal-appearing white matter, and region-matched control spectra from the centrum semiovale were analyzed. The major findings of this study were that in both white matter lesions and normal-appearing white matter in patients with MS, the metabolite ratio NAA/creatine and the total $^{31}$P peak integrals were significantly reduced compared with controls. In addition, in MS lesions NAA/choline and phosphodiester/total $^{31}$P were significantly reduced compared with controls, and in MS normal-appearing white matter there was a trend for NAA/choline to be reduced compared with controls. In normal-appearing white matter in patients with MS, total creatine and phosphocholine were significantly increased compared to controls, as detected with both $^1$H (total creatine peak integrals) and $^{31}$P (phosphocreatine/total $^{31}$P) MRSI techniques. These results suggest reduced neuronal density and altered phospholipid metabolites in white matter lesions in patients with MS. Furthermore, the results suggest the presence of biochemical abnormalities not detected by standard spin echo magnetic resonance imaging in normal-appearing white matter in MS.


Magnetic resonance imaging (MRI) is the neuroimaging procedure of choice for the use in diagnosis of multiple sclerosis (MS) and the detection of MS lesions. In vivo magnetic resonance (MR) spectroscopy single volume techniques have been used to evaluate biochemical changes in white matter lesions in MS. Water-suppressed proton $^1$H MR spectroscopy of the brain yields spectra with three predominant peaks assigned to N-acetyl aspartate (NAA), creatine + phosphocreatine (Cr + PCR), and choline (Cho). Considerable evidence suggests that the amino acid NAA is located almost exclusively in neurons ([1] and references therein). Thus, NAA is a putative marker of neuronal density. Single volume $^1$H MR spectroscopy results have suggested that NAA is reduced in MS lesions [2–11] and that the extent of its reduction may relate to the age of the lesion [2, 3]. It is generally thought that reduction of NAA in MS represents a decrease of axonal density due to some combination of axonal loss and gliosis associated with the demyelinating lesion [2–11].

Phosphorus ($^{31}$P) MR spectroscopy provides spectra...
The PDE resonance general reduction of myelin in MS lesions. Previ-
phodiesters (PDE), PCr, and the three phosphates of with seven resonances corresponding to phospho-
twenty-weighted MRI in patients with MS (termed normal-
ecopy techniques is that they provide little information
ous single volume 31P MR spectroscopy findings of
NAA appearing white matter measured by saturation transfer
MS. A major limitation of single volume 31P MRSI studies. Custom acquisition software and a "home-
operator in quadrature mode were used for the 31P MRSI
The experimental sequence began with acquisition of sagi-
tal and angulated transverse MR images. Sixteen sagittal slices
were obtained to locate the orbital–auditory meatal plane. Su-
accept proton and phosphorus MRSI studies. The subject’s head was securely positioned with the aid of the MRSI laser
MRSI studies conducted with a 2.0-T Gy-
occlusion for MRSI data.
The phosphorus technique (31P echo
The MRI/MRSI Protocol
The MRI/MRSI studies were conducted with a 2.0-T Gy-
were selected to minimize the difference in spatial resolution of the two proton MRSI techniques.
The control group consisted of 19 subjects, ages 23 to 60
whether to use proton and phosphorus MRSI as guides to encompass as much as possible the cen-
plane analysis. The third d

Materials and Methods

Human Subjects
Patients with clinically definite MS, 11 females and 2 males,
ages 23 to 60 years (mean ± SD = 40 ± 11 yr; mean
Kurtzke [28] expanded disability status score (EDSS) = 5.5 ± 2.0; mean Scripps [29] score = 60 ± 20), were selected
if there were multifocal MRI lesions seen on previous MRI
examinations. Patients were stable without evidence of an acute exacerbation at the time of examination. All MS pa-
tients were studied with both 1H and 31P MRSI; one MS
patient, however, was studied with three-dimensional (3D) rather than two-dimensional (2D) 1H MRSI and the results are not included due to the difference in spatial resolution of the two proton MRSI techniques.
The control group consisted of 19 subjects, ages 23 to 60 years (mean ± SD = 36 ± 14 yr), none of whom had
clinical history of neurological disease. Three controls (2 fe-
males, 1 male) were studied with both 1H and 31P MRSI, 3
controls (3 males) were studied with 1H MRSI alone, and 13
controls (7 females, 6 males) were studied with 31P MRSI
alone. All studies were approved by the University of California at San Francisco Human Research Committee and
informed consent was obtained prior to the study.

MRI/MRSI Protocol
The MRI/MRSI studies were conducted with a 2.0-T Gy-
roscan S15 whole body MRI/MRS system (Philips Medical
Systems, Shelton, CT). All MRSI studies were carried out
using gradient phase encoding in two or three spatial dimensions. Standard Philips acquisition software and a saddle-type
86 MHz imaging head coil were used for the imaging and
1H MRSI studies. Custom acquisition software and a "home-
built" inductively coupled high-pass birdcage head coil oper-
at ing in quadrature mode were used for the 31P MRSI
studies. The subject’s head was securely positioned with the aid of a vacuum pack head holder (Olympic Medical, Seattle,
WA) and foam straps with Velcro. Fiducial markings affixed to the subject’s head and placed with the aid of the MRI laser
positioning system enabled accurate repositioning between
proton and phosphorus MRSI studies.
The experimental sequence began with acquisition of sagi-
tal and angulated transverse MR images. Sixteen sagittal slices
(7 mm thick, 0.7 mm gap, TR = 500 msec, TE = 30 msec)
were obtained to locate the orbital–auditory meatal plane. Su-
sequently, 16 transverse slices (7.7 mm thick, 0.8 mm
gap, TR = 2,500 msec, TE = 30, 80 msec) were obtained
accept 20 degrees supraorbitally beyond the orbital–
auditory meatal plane. The MRI gap was used to reduce
acquisition time but resulted in
 accepted space over a sampling scheme
k-space points.

MRSI Protocol
MRSI data were acquired using four-dimen-
sion. For both
ments, two-dimensional multi-
phosphorus determined
spread function voxel size
Spatial filter
Spectroscopy
was used to acquire and spectra.
ated by total
images were produced by 2D integration
ping nature.
tra, complex
PDE were
dowed to a level of bac
was adjust
noise) and
below one

MRSI Spectra
MS lesion
MRSI spettric as guid
only from the
plane analysis. The third d
polated from
interpolation techn
was c (cm) corres
interpolate.
MRSA Processing and Display

MRSA data were processed on a micro-VAX workstation using four-dimensional (4D) Fourier transform reconstruction. For both the proton and phosphorus MRSA experiments, two MRI planes (with gaps) corresponded to one spectral image plane after data reconstruction. A line broadening of 1 Hz was applied to the $^1$H MRSA data and 10 Hz was applied to the $^{31}$P MRSA data. In the spatial dimensions a mild filtering was used with smoothing values of 0.20 exponential multiplication for $^1$H MRSA and 0.25 gaussian multiplication for $^{31}$P MRSA. Zero filling and interpolation were used to obtain $32 \times 32$ display voxels for both proton and phosphorus results. The proton single effective voxel size, as determined from the full width at half height of the point spread function after spatial filtering, was 3 cc. The $^{31}$P effective voxel size, corrected for spherical k-space sampling and spatial filtering, was 27 cc.

Spectroscopic image display software developed in-house was used to display MR images, MR spectroscopic images, and spectra. Total $^1$H and $^{31}$P metabolite images were generated by total spectral integration during reconstruction. Additional images of NAA, Cho + Cr, PME, and PDE were produced during interactive display and analysis by spectral integration over individual resonances. Due to the overlapping nature of the PME–PDE area of the phosphorus spectra, completely separated spectroscopic images of PME and PDE were not possible. Spectroscopic images were windowed to the same level for both MS and control data sets by adjusting brightness and contrast to a consistent color level of background and signal intensities. The background was adjusted to black (essentially filtering the background noise) and the image was adjusted to the color level just below one pixel appearing as white.

MRSA Spectral Selection

MS lesion and normal-appearing white matter $^1$H and $^{31}$P MRSA spectra were selected using the MR image characteristics as guides for spectral selection. Spectra were selected only from the region of the centrum semiovale, the area of largest white matter volume. The 3D phosphorus MRSA plane analyzed was the same as the 2D proton MRSA plane. The third dimension of the phosphorus MRSA data was interpolated from 12 to 16 slices, which corresponds to a 17 cm interpolated phosphorus MRSA slice thickness. The MR protocol was chosen so that two MRI slices with two gaps (17 cm) corresponded to one MRSA slice after interpolation. Data interpolation does not increase resolution, however, and MRSA slices on either side of the two used for MRSA correspondence were also examined to confirm the absence of lesions in the normal-appearing white matter volumes. An outline of a single nominal voxel (defined as the field of view/number of phase encoding gradient steps) was displayed on the MR image. This corresponded to 2.25 cm nominal in-plane resolution for $^{31}$P and 1 cm nominal in-plane resolution for $^1$H. The nominal voxel is smaller than the effective voxel because it does not take into account the effects of spatial filtering and reduced k-space sampling described above. Only voxels wherein either normal-appearing white matter entirely occupied the voxel or MS lesions predominantly occupied the voxel were selected. One to five entirely normal-appearing white matter voxels and one to five predominantly lesion voxels were selected per subject, depending upon the degree of lesion involvement. Proton MS spectra analyzed from 12 subjects included 48 lesion spectra and 37 normal-appearing white matter spectra. Phosphorus MS spectra analyzed from 13 subjects included 39 lesion spectra and 30 normal-appearing white matter spectra. There were fewer $^{31}$P spectra due to the lower spatial resolution compared with $^1$H MS.

Control and MS spectra were region-matched due to trends for regional differences in controls (unpublished results). A total of 59 proton and 191 phosphorus control spectra were selected from the control subjects. Selection of periventricular spectra increased the probability of cerebrospinal fluid (CSF) contamination within the voxel, which would reduce the peak integrals but not affect the metabolite ratios because the metabolites evaluated are not present in CSF to a detectable degree. Only spectra with NAA or PCr signal-to-noise ratios greater than 10 were utilized for analysis.

MRSA Spectral Analysis

All spectra were curve fitted by a least-squares method (NMRS program, New Methods Research, Inc, Syracuse, NY) to generate peak integrals. Proton magnitude spectra were curve fitted with gaussian line shapes to provide peak integrals for NAA, Cr, Cho, and total (non-water) $^1$H. Greater error is introduced when complex, rather than magnitude, proton MRSA data are used due to the phase distortion errors that arise from magnetic field inhomogeneities in the saddle-type proton coil used. The residual after curve fitting was used as the criterion for goodness of fit. It is our experience that magnitude proton spectra have the least residual from curve fitting when gaussian, as opposed to Lorentzian, line shapes are used. An external standard that provides coil loading corrections was not acquired with the proton MRSA data; however, control and MS proton data were acquired with the same pulse length and mean power requirements. Thus, peak integrals are given for the proton metabolites, although it is recognized that spectrometer instabilities may affect the peak integrals. Metabolites are also reported as ratios to account for the effects of coil loading and signal relaxation.

Phosphorus complex spectra were processed with a 300 Hz convolution difference filter to remove the broad baseline that presumably arises from the less mobile phospholipids [12–14]. The resulting $^{31}$P spectrum was curve fitted with gaussian line shapes, except for PCr, which was fitted with a

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Results
Proton MRSI
Figure 1 shows typical proton MRSI measurements of a 33-year-old female control (Fig 1A) and a 39-year-old woman with MS and an EDSS score of 8.0 (Fig 1B). Shown are MR images with the PRESS selected volume of interest in blue, and spectroscopic images of NAA and Cho + Cr in gray scale with MRI outlines in red to demonstrate the anatomical location of the MRSI metabolite distributions. Large hyperintense areas of MS lesions are noted on the MS MR image (see Fig 1B). The lesion outlines are seen on the red MRI outline. The NAA spectroscopic image shows a decrease of the NAA signal within the area of the MS lesions. The lack of deficits of Cho + Cr within the lesion areas indicates that NAA deficits were not due to contamination from the ventricles; CSF contains no detectable NAA, Cr, or Cho.

Table 1 provides mean NAA/Cr and NAA/Cho metabolite ratios as well as peak integrals for NAA, Cr, Cho, and total (non-water) $^1$H for control subjects and patients with MS. In MS white matter lesions NAA/Cr, NAA/Cho, the NAA peak integral, and total $^1$H were significantly reduced, and the peak integrals of Cr and Cho were significantly elevated compared to controls. In MS normal-appearing white matter NAA/Cr was significantly reduced, the Cr + PCr peak integral was significantly elevated, and there were trends for NAA/Cho and the NAA peak integral to be reduced compared to controls. While the NAA, Cr, and Cho peak integrals were not corrected for coil loading and relaxation, the peak integral values reflect the changes in the individual components of the metabolite ratios and indicate that elevations of Cr and Cho signals contribute to the reductions in NAA/Cr and NAA/Cho.

Phosphorus MRSI
Figure 2 shows the phosphorus MRSI results from a control subject (Fig 2A) and a patient with MS (Fig 2B). MR images and spectroscopic images of total $^3$P, PME (phosphomonoesters), and PDE (phosphodies-esters) are displayed. Control metabolites appear to be bilaterally symmetrically distributed between the two cerebral hemispheres. By contrast the MS patient showed reduced PME and PDE in lesion and normal-appearing white matter regions. All MS patients studied showed asymmetries in the distributions of PME and PDE, independent of the amount and distribution of lesions detected with spin echo MRI (see Fig 2B). Figure 2C shows decreases in PME and PDE in spectra from MS lesion and normal-appearing white matter compared with control.

Table 2 shows that the ratio PDE/total $^3$P was significantly reduced in MS white matter lesions compared to controls, and PCr/total $^3$P was significantly increased in MS normal-appearing white matter compared to controls. Peak integrals indicated 32 to 41% reductions in total $^3$P signal intensities in both white matter lesions and normal-appearing white matter in MS (see Table 2). Phosphorus measurements of an external hexamethylphosphoroustramide standard indicated coil loading variations of less than 10% between subjects, and there was no difference in phosphorus coil loading between the control data and MS data. Thus, the phosphorus peak integrals reflect substantial reductions of total $^3$P signals in MS lesions and normal-appearing white matter compared to controls.

Discussion
The major findings of this study were that in both white matter lesions and normal-appearing white matter in patients with MS, the metabolite ratio NAA/Cr and the total $^3$P peak integrals were significantly reduced compared to controls. In addition, in MS white matter lesions NAA/Cho and PDE/total $^3$P were significantly reduced compared to controls, and in MS normal-appearing white matter there was a trend for NAA/Cho to be reduced compared to controls. In normal-appearing white matter in patients with MS, total Cr and PCr were significantly increased compared to controls, as detected with both $^1$H (Cr + PCr peak integrals) and $^3$P (PCr/total $^3$P) MRSI techniques.

The observed reductions of NAA/Cr and NAA/Cho in MS white matter lesions confirm previous re-
Fig. 1. Two-dimensional magnetic resonance spectroscopic imaging (MRSI) and magnetic resonance imaging (MRI) measurements displayed on a transverse magnetic resonance image (TRUTE = 2,500 ms) and spectrometric images of N-acetylaspartate (NAA) and choline plus creatine (Cho + Cr) shown in gray scale with magnetic resonance image (MRI) outline overlaid in red. Superimposed on the MRI is the PRESS selected volume of interest (10 x 10 x 2 mm) outlined in blue. Typical regions from which spectra were collected are indicated by circular voxel outlines. Images at right are coronal slices corresponding to the spatial region/voxel outlined for data analysis.

A 33-year-old female control subject: the NAA, Cho, and Cr distributions are nearly uniform throughout the volume of interest. (B) A 39-year-old woman with multiple sclerosis (MS) lesion outlines are seen on the MRI outline. Lack of NAA signal in MS lesion outlines is evident within the MS lesion area. Lack of detection of Cho + Cr are noted within the MS lesion area. Lack of NAA signal in MS lesion outlines is noted in both MS lesion and normal-appearing white matter in MS compared to controls.
Table 1. Proton MRSI Metabolite Ratios and Peak Integrals for Control White Matter, MS Normal-appearing White Matter, and MS Lesions

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 59 spectra from 6 subjects)</th>
<th>MS NAWM (n = 37 spectra from 12 subjects)</th>
<th>MS Lesions (n = 48 spectra from 12 subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>3.2 ± 0.1</td>
<td>2.8 ± 0.1*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>NAA/Cho</td>
<td>2.5 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>Integrals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>0.44 ± 0.01</td>
<td>0.43 ± 0.01</td>
<td>0.34 ± 0.01*</td>
</tr>
<tr>
<td>Cr</td>
<td>0.14 ± 0.00</td>
<td>0.16 ± 0.01b</td>
<td>0.16 ± 0.01b</td>
</tr>
<tr>
<td>Cho</td>
<td>0.19 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.24 ± 0.01*</td>
</tr>
<tr>
<td>Total 1H</td>
<td>0.77 ± 0.02</td>
<td>0.79 ± 0.03</td>
<td>0.74 ± 0.02b</td>
</tr>
</tbody>
</table>

Data given as mean ± SEM. Integrals are peak areas determined from spectral curve fitting, expressed in arbitrary units. Total 1H (non-water) is the sum of NAA, Cr, Cho.

*P < 0.05, corrected for multiple comparisons (to test major hypotheses regarding alterations in neuronal density and phospholipid metabolism).

P < 0.05, uncorrected for multiple comparisons (to test for trends).

MRSI = magnetic resonance spectroscopic imaging; MS = multiple sclerosis; NAWM = normal-appearing white matter; NAA = N-acetyl aspartate; Cr = creatine; Cho = choline.

Results obtained using single volume proton magnetic resonance spectroscopy [2-11]. Reduced NAA does not necessarily indicate a reduction in the number of neurons because such a change might also be due to decreased concentration of NAA in axons, gliosis resulting in diminished density of neurons, or changes in relaxation times. Proton peak integrals, while not corrected for coil loading, indicated that increases of Cr and Cho also contributed to the observed reductions of NAA/Cr and NAA/Cho in MS lesions (see Table 1). Reduced PDE/total 31P in MS lesions suggests decreased phospholipids and/or phospholipid metabolites but could also represent increased T1 or decreased T2 relaxation times due to alterations in molecular mobility. Fifty percent of myelin lipids are phospholipids and lipids are reduced in MS lesions, largely a result of myelin loss [15]. Our in vivo findings of reduced PDE/total 31P in MS lesions are consistent with such in vitro chemical analyses.

The important new finding of this study was similar, but smaller, reductions in NAA/Cr, NAA/Cho, and total 31P in normal-appearing white matter in MS. Significant increases in PCr/total 31P and Cr + Pcr 1H peak integrals were also detected in normal-appearing white matter, suggesting an alteration of energy metabolites in MS normal-appearing white matter. In addition to the above reasons, the reduced NAA in MS normal-appearing white matter could also represent loss of axons due to a previous lesion at that site or to Wallerian degeneration from a lesion at another location. In contrast to the minor changes of 1H peak integrals in MS normal-appearing white matter, 31P peak integrals indicated significant reductions in total 31P metabolites in MS normal-appearing white matter. Use of the total 31P ratio suggested smaller changes in the phosphorus metabolites than the peak integrals indicated due to the magnitude of the decrease in total 31P for both MS lesions and MS normal-appearing white matter (see Table 2). Relaxation measurements would determine the extent to which changes in relaxation, as opposed to changes in metabolite concentrations, contribute to the altered MRSI signal intensities in MS.

Considerable evidence suggests that there may be an inherent biochemical defect in MS normal-appearing white matter [20-27, 31-46]. Although many of these biochemical studies on macroscopically normal white matter have inadequate histological control, Allen and colleagues [31, 32], in carefully performed studies of macroscopically normal white matter in postmortem samples of patients with MS, found marked astrocytic proliferation and increased lysosomal β-glucosaminidase in regions completely devoid of perivascular inflammation. Astrogliosis throughout the entire white matter, as evaluated by increases in glial fibrillar acidic protein, was also reported by others [33]. Furthermore, the observation of higher levels of DNA in MS normal-appearing white matter compared to controls confirmed histological reports of gliosis [34]. Other reported abnormalities of normal-appearing white matter include general reduction of phospholipids [35-37], 35% decrease in myelin yield from sucrose gradient extraction [38], reduced myelin lipid phase transition temperature, suggesting differences in the physical organization of the myelin lipid bilayer [39], alterations of myelin membrane proteins and possibly of glial proteins [40], reduction in myelin basic protein [41], increased protein enzyme activities...
Fig 2. $^{31}$P three-dimensional magnetic resonance spectroscopic imaging measurements displaying angulated transverse magnetic resonance image (TR/TE = 2,500/30 msec) and spectroscopic images of total $^{31}$P, phosphomonoesters (PME), and phosphodiesters (PDE). In this color presentation red represents higher concentration and blue represents lower concentration. Typical regions from which spectra were selected are indicated by circular voxel outlines. (A) Control: metabolites are symmetrically distributed. (B) Moderate areas of periventricular lesions: 40-year-old woman with multiple sclerosis (MS). Selection of periventricular lesions increases the probability of cerebrospinal fluid (CSF) contamination within the voxel, which would reduce the SIN but not alter the metabolite ratios. $^{31}$P metabolites are not present in CSF to a detectable degree. Metabolites are asymmetrically distributed in spectroscopic images. PME and PDE reductions are noted in both lesion and normal-appearing white matter regions. (C) Single voxel (27 cc) $^{31}$P spectra from control centrum semiovale white matter, MS normal-appearing white matter, and MS lesion. Peak assignments are labeled. Both PME and PDE are reduced in the MS lesion and normal-appearing white matter spectra compared to control.
The presence of perivascular lymphocytes and macrophages [44], the presence of large numbers of T cells [45], and increased FAS RNA accumulation [46]. The current findings of 1H and 31P metabolite alterations in normal-appearing white matter in MS are consistent with these previously reported results.

It is possible that some or all of the MRSI findings in normal-appearing white matter reflect inflammation and lesions that are below the spatial resolution of MRI [47]. It is possible that the gap between MRI slices may have obscured detection of small MS lesions. It is also possible that inclusion of some degree of lesion in the voxels selected from normal-appearing white matter, due to contamination from surrounding voxels, could result in alterations of metabolites in normal-appearing white matter in MS. Several points, however, argue against contamination as an explanation for the metabolite alterations in normal-appearing white matter in MS. First, metabolite alterations were observed in normal-appearing white matter of MS brains with few lesions (see Fig 2B). Second, normal-appearing white matter changes in MS were detected with 1H MRSI in which the voxel size is small and there is less risk of tissue contamination. Third, all metabolites in normal-appearing white matter in MS did not show the same degree of change. For example, in MS normal-appearing white matter NAA was decreased and Cho was unchanged, whereas in MS lesions NAA was decreased and Cho was increased. Therefore, although it is possible that inclusion of lesion tissue may contribute to the metabolite alterations in normal-appearing white matter in MS, this explanation is unlikely.

In conclusion, the ability of proton and phosphorus MRSI to demonstrate abnormalities in white matter areas that appear normal on MRI in patients with MS suggests that this technique is a more sensitive measure of the biochemical changes that occur in MS than standard spin echo MRI. Until the current findings are replicated, however, the results should be interpreted with caution. Nevertheless, such MRSI changes may reflect an early stage of a process that leads to acute or chronic demyelination, and thus might provide an important tool for the in vivo biochemical evaluation of the natural history of the disease.

This investigation was supported, in part, by a postdoctoral fellowship from the National Multiple Sclerosis Society (C.A.H.) and by NIH grants RO1-DK33293 (M.W.W.), R01-CA48815 (A.A.N.), HL07192 (J.W.H.), and 5ROlMH45680 (G.F.). Philips Medical Systems, and the Department of Veterans Affairs Medical Research Service (M.W.W.).

We thank Dr J. M. Constans for his assistance with data analysis.

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Table 2. Phosphorus MRSI Metabolite Ratios and Peak Integrals for Control White Matter, MS Normal-appearing White Matter, and MS Lesions

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 191 spectra from 16 subjects)</th>
<th>MS NAWM (n = 30 spectra from 13 subjects)</th>
<th>MS Lesions (n = 39 spectra from 13 subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME/total 31P</td>
<td>0.16 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>PDE/total 31P</td>
<td>0.40 ± 0.00</td>
<td>0.39 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>PCR/total 31P</td>
<td>0.18 ± 0.00</td>
<td>0.21 ± 0.01c</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Pi/total 31P</td>
<td>0.06 ± 0.00</td>
<td>0.06 ± 0.00</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>γ-ATP/total 31P</td>
<td>0.20 ± 0.00</td>
<td>0.20 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>α-ATP/total 31P</td>
<td>0.18 ± 0.00</td>
<td>0.19 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>β-ATP/total 31P</td>
<td>0.13 ± 0.00</td>
<td>0.13 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Total 31P</td>
<td>2.321 ± 109</td>
<td>1.571 ± 46b</td>
<td>1.371 ± 49b</td>
</tr>
</tbody>
</table>

Data given as mean ± SEM. Total 31P is the sum of the peak integrals of PME, Pi, PDE, PCR, γ-ATP, α-ATP, and β-ATP.

*p < 0.05, corrected for multiple comparisons (to test major hypotheses regarding alterations in neuronal density and phospholipid metabolism).

b* < 0.001, uncorrected for multiple comparisons (to test for trends).

MRSI = magnetic resonance spectroscopic imaging; MS = multiple sclerosis; NAWM = normal-appearing white matter; PME = phospho-monoesters; PDE = phosphodiester; PCR = phosphocreatine; Pi = inorganic phosphate; ATP = adenosine triphosphate.

[34, 42, 43], the presence of perivascular lymphocytes and macrophages [44], the presence of large numbers of T cells [45], and increased FAS RNA accumulation [46]. The current findings of 1H and 31P metabolite alterations in normal-appearing white matter in MS are consistent with these previously reported results.

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