Effects of Chronic Alcohol Consumption on the Broad Phospholipid Signal in Human Brain: An In Vivo $^{31}$P MRS Study

M. R. Estilaei, G. B. Matson, G. S. Payne, M. O. Leach, G. Fein, and D. J. Meyerhoff

Background: Phosphorus magnetic resonance spectroscopy ($^{31}$P MRS) allows for the measurement of phospholipids and their breakdown products in the human brain. Fairly mobile membrane phospholipids give rise to a broad signal that co-resonates with metabolic phosphodiester signals. Chronic alcohol exposure increases the rigidity of isolated brain membranes and, thus, may affect the amount and transverse relaxation times ($T_2$) of MRS-detectable phospholipids. We tested the hypothesis that subjects who were heavy drinkers have stiffer membranes than controls who were light drinkers, as reflected in a smaller broad signal component and a shorter $T_2$ of the broad signal in $^{31}$P MR spectra of the brain.

Methods: Thirteen alcohol-dependent heavy drinkers (mean age 44 years) were studied by localized $^{31}$P MRS in the centrum semiovale and compared with 17 nondependent light drinkers of similar age. The broad component signal was separated from the metabolite signal by convolution difference, which is based on the large difference in line widths of these two signals. Longitudinal and $T_2$ relaxation times were measured using standard methods.

Results: The broad component integral was 13% lower in the brain of heavy drinkers compared with light drinkers ($p < 0.001$) and remained significantly smaller after corrections for both longitudinal and transverse relaxations ($p < 0.01$). The $T_2$ distribution of the broad component consistently showed two resolvable components in both groups. The fast relaxing component had the same $T_2$ in both groups ($T_2 = 1.9$ msec). The slower relaxing component $T_2$ was 0.6 msec shorter in heavy drinkers compared with light drinkers ($p = 0.08$).

Conclusions: These results, observed in the absence of white matter volume loss, are consistent with biochemical alterations and higher rigidity of white matter phospholipids associated with long-term chronic alcohol abuse. The observed smaller broad signal component in these relatively young heavy drinkers is a sensitive measure of white matter phospholipid damage.

Key Words: Magnetic Resonance Spectroscopy, Brain, Phospholipid, Myelin, Relaxation.
using electron spin resonance (ESR) and fluorescence polarization (FPZ) techniques. Although there is general consensus about ethanol's effects on membrane fluidity, the physiologic and functional importance of increased membrane rigidity after chronic alcohol exposure is a matter of discussion (e.g., Deitrich et al., 1989; Tan and Weaver 1997). Most studies have been performed in vitro with relatively high ethanol concentrations, which are not physiologically relevant. Nevertheless, because it is generally accepted that membranes in vivo are more sensitive to ethanol effects (e.g., Harris et al., 1984c) and because the membrane fluidity hypothesis of alcohol action has not been disproved, any studies that can shed light on alcohol-induced membrane changes are potentially important, especially if they can be performed in humans in vivo. The ESR and FPZ methods determine membrane fluidity by introducing compounds into specific regions of isolated membranes and observing the behavior of these compounds. A major disadvantage of these methods is that they report only the characteristics of the region in which their probes are located, which may not be representative of the membrane as a whole. In addition, membranes prepared in vitro may not adequately reflect the heterogeneous environment of the brain in vivo and are often specific to the method of preparation (Rowe, 1992). Similar studies in animals are problematic because sedation may affect the membranes under examination (Altura et al., 1992; Denays et al., 1993). Moreover, they may not be completely relevant to humans because it has been suggested that some effects of EtOH on the brain are species dependent (Petroff et al., 1990).

The chronic effects of EtOH on membranes can be characterized in vivo by phosphorus-31 magnetic resonance spectroscopy ($^{31}$P MRS) of the human brain. This method detects the phosphate head groups in phospholipids, which constitute about 60% of total brain lipid, and is sensitive to the amount and rigidity of lipid. In vivo $^{31}$P MR brain spectra obtained at low magnetic fields (1.5–2 Tesla) contain a large resonance contribution from phosphodiester (PDE) (see stylized spectra in Fig. 1). The three major signal components that contribute to this spectral region (Kilby et al., 1991) are: (1) narrow resonances from PL deacylation products such as glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE) (Bárány et al., 1985) with an approximate line width of 1ppm; (2) broad resonances from fairly mobile PL in membrane bilayers and vesicles with a line width-at-half-height of 15 to 20 ppm (Cerán et al., 1986; McNamara et al., 1994; Murphy et al., 1989); and (3) signal from motion-restricted PL in membrane bilayers (Kwee and Nakada, 1988) with a line width of about 50 ppm. It has been shown that, at low magnetic field, about 92% of the total PL content of excised rat brain is visible by $^{31}$P MRS methods (Kilby et al., 1991). Corresponding methods applied to human brain are likely to detect a lower percentage of the signal from PL because of experimental constraints associated with local-

**Fig. 1.** Stylized $^{31}$P MR spectra from human brain with (top) and without (bottom) the broad component (BC) representing signal from membrane PL (see text). The narrow resonances represent phosphomonoesters (PME), inorganic phosphate (Pi), metabolic phosphodiesters (PDE), phosphocreatine (PCr), and γ-, α-, and β-ATP, from left to right.
MR Measurements

All measurements were carried out on a whole body 1.5 T Magnetom Vision system (Siemens Inc., Iselin, NJ) equipped with a homemade double tuned (31P/1H) birdcage coil operating at 25.8 MHz for 31P (Mason et al., 1999). To minimize motion of the subject's head, a vacuum-molded head holder (Vac-Pac, Olympic Medical, Seattle, WA) was used. The MRI protocols consisted of sagittal TI-weighted localizer scans (TR/TE = 255/6 msec) and axial turbo spin-echo scans (TR/TE = 7000/1485 msec) angled along an imaging line connecting the anterior and posterior commissures. These images were used to locate a volume of interest (VOI) above the ventricles in the centrum semiovale for 31P MRS. Figure 2 shows the typical location of the VOI (70 x 100 x 24 mm3) on sagittal and axial images. The VOI contained predominantly white matter tissue and was placed carefully to avoid inclusion of ventricles.

An ISIS sequence (Image-Selected-In vivo-Spectroscopy) (Ordidge et al., 1986) with selective 180-degree hyperbolic secant pulses for accurate inversion (7.68 msec long) was used for localization of the VOI. The ISIS pulse sequence was followed by a spin echo acquisition with TE values ranging between 0.6 and 10.5 msec and a nonselective 280 μs long refocusing pulse. Each spectrum was acquired with TR = 2000 msec, a spectral bandwidth of 4000 Hz, 1024 data points, and 256 msec acquisition time to result in a total measurement time of 270 sec.

**BC Integral**

To obtain the BC integral, ISIS-localized 31P spectra were acquired with TR/TE = 2000/0.6 msec and processed using NMRl (New Methods Research, Inc., St. Louis, MO) software. The rater (MRE) was blinded to the subjects' group assignment. The automated processing procedures included the following main steps: (1) A Lorentz-Gaussian transformation (with a ~10 Hz exponential filter and a 20 Hz Gaussian filter) was used for apodization. (2) BC spectra were obtained using the convolution difference method (Roth and Kimber, 1982), which is given by:

\[ SC_D = S(1 - e^{-\alpha t}) \]

\[ BC = S - SC_D \]

Here, S is the measured raw signal intensity, SC_D is the remaining signal after the subtraction of BC from the measured signal, f is the convolution-difference factor, and L is an exponential filter with 150 Hz Lorentzian broadening. The convolution factor f represents the fraction of BC contributing to the acquired signal at a given TE and was 0.85 for spectra at TE = 0.6 msec. (3) The integral of BC was determined by fitting the asymmetric broad spectrum to multiple Gaussian lines, minimizing residuals, and summing over all the Gaussian-line integrals. Typically six Gaussian lines were placed in the broad component spectrum at the onset of the automatic fitting routine at the signal maximum and at shoulders to the left and right of the maximum. The NMRl program was then allowed to vary the frequency position, line width, and amplitude of each individual Gaussian peak to yield an overall fit solution with minimum residual. This procedure took between 5 and 20 sec. The residuals after curve fitting were used as the criterion for goodness of the BC fit. It is important to note that these six Gaussian lines do not represent any specific resonances. This approach was used solely to determine the total integral of the BC spectrum accurately.

**Relaxation Times**

\( T_2 \) Relaxation Measurements. To determine T2 values of BC, ISIS spectra (TR = 2000 msec) were obtained with 8 TE values (0.6, 1.5, 2.5, 3.5, 5, 6.5, 8, and 10.5 msec) from 12 HD and 15 LD subjects. TD-dependent convolution factors (f) varied between 0.85 and 0.6 and were kept constant for a given TE and for all subjects irrespective of group assignment. This range was determined empirically to provide a spectrum, at a given TE, with a relatively flat baseline. The factors were derived from 6 of the 30 data sets of this study (HD and LD subjects) by a rater (DJM) cognizant of the group assignment. No significant group differences were...
Statistics

Statistics were performed using the 'proc GLM' routine in SAS™ software (SAS Institute Inc., Cary, NC). Signal integrals and relaxation times were tested for group differences using ANOVA. The differential effects of family history of alcoholism (FH+ or FH-) and group membership (HD or LD) on MR measures were assessed using a 2x2-factorial design. Linear relationships of MR versus drinking measures and age were assessed using Pearson correlations. Data are reported as mean ± one standard deviation and results were considered significant at the 0.05 level.

RESULTS

BC Integral

Figure 3a shows a representative 31P MR spectrum at the shortest possible TE of 0.6 msec with the broad component present under the narrow resonances. These resonances represent (from left to right) the phosphomonooesters (PME) such as phosphocholine and phosphoethanolamine, inorganic phosphate (Pi), the phosphodiesters (PDE) such as glycerophosphocholine and glycerophosphoethanolamine, phosphocreatine (PCr), and γ-, α-, β-ATP. Figure 3b shows the same spectrum after applying the convolution difference method described previously. Subtraction of these two spectra yields the spectrum of BC (Fig. 3c). Table 1 lists the integral of BC and metabolite resonances for LD and HD with no relaxation corrections (see column 2). A 13% smaller area of BC was observed in HD (p = 0.0005). No significant differences were observed for any of the metabolite integrals (see Table 1) (all p > 0.37). There was no main effect of FH (p = 0.43) and no FH-by-group interaction effect on the BC integral (p = 0.52).

Relaxation Times

T2 Relaxations. T2 determinations were performed for two reasons, to obtain information on the rigidity of PL, and to correct the integral of BC for T2 relaxation. Figure 4 shows the BC as a function of TE. Each spectrum spreads approximately between -30 and +30 ppm with a line width at half height around 18 ppm. Figure 5 shows a typical T2 decay curve from BC plotted on a semilogarithmic scale. It clearly deviates from a single straight line, indicating its multiexponential nature. Table 2 lists T2 values for the long and short T2 components along with their respective am-

\[ S(t) = S_1 e^{-t/T_1} + S_2 e^{-t/T_2} \]

where S(t) is the total magnetization at a given time and S1 and S2 are the equilibrium magnetizations for the slow and fast decaying components. T1 and T2 are the spin-spin relaxation times for the long and short T2 components, respectively. Different biexponential fitting routines (as well as one multiexponential method) yielded very similar values for the short T2 component, but indefinite or widely varying long T2 values. By employing a model that assumes T2 ≠ T2 (Kilby et al., 1990) we eliminated the uncertainty in the determination of the long T2. Using this assumption, it can be shown that when TE ≪ T2,

\[ \ln(S(TE)) = \ln(S_1 + S_2) - \frac{S_1}{S_2 + S_1} \times \frac{TE}{T_2} \]

and when TE ≫ T2,

\[ \ln(S(TE)) = \ln(S_2 - \frac{TE}{T_2}) \]

Linear regression fits to the four shortest TE data sets, using Eq. 4, and fits to the four longest TE data sets, using Eq. 5, allowed determination of relative magnetization and T2 for the fast and slow relaxing components, respectively. T2 values measured this way for each subject were used to apply individual T2 corrections for BC. We also calculated T2 from just the three longest TE data sets to better satisfy the assumption of TE ≫ T2.

T2 Relaxation Measurements. To correct the signal intensity of BC for a possible T1 relaxation effect, T1 values were obtained from five HD subjects and six LD subjects. A standard fast inversion-recovery sequence was used for T1 determination. The pulse sequence consisted of a hard 180° pulse (280 μs long) followed by the ISIS sequence to obtain T1 magnetization-decay curves from the localized VOI. Seven inversion time (TI) values (10, 180, 280, 1000, 1300, 1700, and 2500 msec) were selected to optimize the signal-to-noise ratio (S/N) for the BC spectrum and T1 determination. The relaxation delay was kept constant at 1990 msec for all TI. A three-parameter fit to the decay curve was used to derive T1. Because T1 was not determined for each subject, the average T1 value obtained for a group was used to correct each individual's BC for T1 effects. Due to low S/N, T1 analysis was not carried out for the narrow resonances.
Table 1. Integral of BC and Metabolites With and Without Relaxation Corrections

<table>
<thead>
<tr>
<th></th>
<th>BC</th>
<th>BC $T_2$ corrected</th>
<th>BC $T_1$ &amp; $T_2$ corrected</th>
<th>PDE</th>
<th>PME</th>
<th>Pi</th>
<th>PCr</th>
<th>$\gamma$-ATP</th>
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<tr>
<td>LD</td>
<td>82.7 ± 5.1</td>
<td>99.5 ± 7.3</td>
<td>110.0 ± 8.0</td>
<td>11.7 ± 1.7</td>
<td>3.1 ± 0.7</td>
<td>2.6 ± 0.6</td>
<td>4.0 ± 0.5</td>
<td>4.8 ± 1.2</td>
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<tr>
<td>HD</td>
<td>72.0 ± 9.4</td>
<td>86.3 ± 12.0</td>
<td>97.7 ± 13.8</td>
<td>11.1 ± 2.1</td>
<td>2.9 ± 0.7</td>
<td>2.6 ± 0.9</td>
<td>4.1 ± 0.7</td>
<td>4.3 ± 1.0</td>
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<tr>
<td>$\rho$</td>
<td>0.0005</td>
<td>0.002</td>
<td>0.007</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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All values mean ± 1 SD. Integrals reported in arbitrary units.
HD: $n = 13$; LD: $n = 17$. NS, not significant.

Fig. 4. BC spectra plotted as a function of TE. The BC signal resonates between about −30 and +30 ppm with a line width at half height of approximately 18 ppm or 465 Hz.

BC Integral Corrected for Relaxation Effects

Table 2 also lists the integral of BC after $T_1$ and $T_2$ relaxation corrections (see columns 3 and 4). As stated earlier, the $T_2$ correction decreased the raw BC integral difference between LD and HD insignificantly. Applying both $T_1$ and $T_2$ corrections decreased the mean BC integral difference from 13 to 11%. Nevertheless, the relaxation-corrected BC integral remained significantly lower in HD compared with LD ($p = 0.007$).

MRI Segmentation

To test whether less white matter in the $^{31}$P MRS VOI may be responsible for the lower BC in HD, we segmented the MRI's of 10/17 LD and 11/13 HD subjects into gray matter, white matter, sulcal, and ventricular cerebral spinal fluid (CSF) using published methods (Goldman et al., 1999; MacKay et al., 1996; Tanabe et al., 1997). After covariation for intracranial volume and age, no group differences were found for white matter, gray matter, ventricular, or sulcal CSF when analyzing the whole brain (all $p > 0.3$) or when restricting analyses to a smaller supraventricular brain region that included and surrounded the $^{31}$P MRS VOI (all $p > 0.2$). The smaller brain region that was obtained by adding segmented Talairach brain regions had an average volume of 330 ml, encompassing the nominal 168 ml VOI (note that the effective VOI size is larger due to nonideal pulse shapes). Restricting spectroscopic analyses to the smaller sample of subjects who also had segmented MRI's, retained significantly lower BC in the HD (73.1 ± 9.0) compared with LD group (82.6 ± 5.3, $p = 0.02$) and increased the statistical significance of shorter $T_2$ in the HD (7.1 ± 0.9 msec) compared with LD group (7.9 ± 1.1)
msec, \( p = 0.06 \). Therefore, our spectroscopic findings are not due to less white matter in the VOI of HD subjects.

**Associations of BC Signal With Age, Drinking Measures, or Family History**

There were no significant correlations of the BC signal integral or its relaxation times with age, years of abuse, average monthly drinks, or total lifetime consumption in either group or in both groups combined. A positive family history of alcohol abuse did not predict a smaller BC integral or shorter relaxation times. In either group, scatter plots of the data showed that measures in FH-positive subjects were not distinguishable from measures in FH-negative individuals.

**DISCUSSION**

Phospholipids (PL) constitute more than 60% of total brain lipid, most of which are incorporated in membranes. In brain membranes, the bulk of PL is in bilayers and consists of constituents such as phosphatidylcholine, phosphatidylserine, sphingomyelin, phosphatidyl-ethanolamine, and phosphatidylinositol. Some PL in bilayers are motion-restricted, and, because of their resultant short \( T_2 \), a small fraction of them may not be visible to standard in vivo MRS methods. It is the PL in vesicles, nonbilayer structures, and fairly mobile membrane bilayers that give rise, collectively, to the asymmetric BC signal in vivo \(^{31}\)P MR spectra obtained at relatively low magnetic field strengths. Very few human studies have measured and/or characterized this component. For example, the BC integral was reduced nearly 35% in lesions and in normal appearing white matter in patients with multiple sclerosis compared with controls, suggesting reduced myelin PL concentration or altered relaxation times (Husted et al., 1994). In most studies, however, this broad resonance has been ignored, excluded from spectra (Christensen et al., 1996; MacKay et al., 1993; Meyerhoff et al., 1995), or simply was not present at high magnetic field strengths (3-5 T) because of broadening of the phospholipid signal beyond MR spectroscopic detection (e.g., Murphy et al., 1989; Sauter et al., 1987).

The degree to which chronic heavy drinking affects the total amount, rigidity, and the composition of brain membrane PL has not been evaluated using \(^{31}\)P MRS at low magnetic fields. In a previous study (Denays et al., 1993), \(^{31}\)P MRS was used to monitor high-energy phosphate metabolites in rats at 4.7 T. The primary focus of that study was to investigate the effect of acute and chronic ethanol exposure on narrow line resonances in \(^{31}\)P spectra. The BC contribution to spectra was essentially nonexistent under those experimental conditions, and the broad resonance from bone was removed during data processing. In contrast, our in vivo human brain studies allow observation of bulk PL membrane changes that may be associated with chronic alcohol abuse and contribute unique human data to the discussion of the membrane fluidity hypothesis in alcohol research.

**BC Integral**

The significantly lower BC integral in HD compared with LD subjects described here is qualitatively consistent with a previous report of a decrease in the total mass of PL in rat-brain homogenate examined after chronic ethanol treatment (Vrbaski et al., 1984), but it is inconsistent with other animal studies reporting elevation (Rawat, 1974) or no change (Alling et al., 1982; Doyle et al., 1990; Gustav-son and Alling, 1989; Wood et al., 1993) in the total mass of PL and cholesterols. Our data suggest that the concentration of any or all of the components contributing to the BC described above was reduced and that their \( T_2 \) relaxation times were shortened. Because the measured \( T_2 \) changes accounted for only 1% decrease in BC integral, our primary result is a reduction in the concentration of PL in HD subjects. Studies on juvenile and adult gerbils show that the major fraction of the asymmetric broad resonance originates from myelin (Kanashiro et al., 1990). If this applies to humans, it suggests that the observed decrease in BC integral may be due to loss of myelin in the HD group. However, loss of other phosphorus containing membrane components may contribute to this decrease as well. Whatever the molecular moiety responsible for the observed smaller BC integral in HD, it could provide an explanation for the white matter tissue-volume loss often described in pathologic (Kril et al., 1997) and structural neuroimaging studies of chronic alcoholics of mean age greater than 45 years (Pfefferbaum et al., 1995; Shear et al., 1994). It should be noted, however, that MRI segmentation of a subsample of our study subjects revealed no differences in the amount of tissue types between HD and LD subjects (whole brain and supraventricular brain region encompassing the \(^{31}\)P MRS VOI). Therefore, in this relatively young sample, less white matter tissue in the VOI cannot explain the lower BC in HD compared with LD subjects. We conclude that the observed lower BC in these relatively young HD is a sen-

<table>
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<tr>
<th></th>
<th>( S_i ) [a.u.]</th>
<th>( S_2 ) [a.u.]</th>
<th>( T_{2i} ) [msec]</th>
<th>( T_{2r} ) [msec]</th>
<th>( ^{*} T_{1} ) [msec]</th>
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<tbody>
<tr>
<td>LD</td>
<td>61.5 ± 9.8</td>
<td>37.8 ± 6.1</td>
<td>1.9 ± 0.2</td>
<td>7.7 ± 1.3</td>
<td>858 ± 81</td>
</tr>
<tr>
<td>HD</td>
<td>49.1 ± 11.5</td>
<td>37.1 ± 5.7</td>
<td>1.8 ± 0.3</td>
<td>7.1 ± 0.9</td>
<td>932 ± 84</td>
</tr>
<tr>
<td>( p )</td>
<td>0.003</td>
<td>NS</td>
<td>0.08</td>
<td>0.08</td>
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</table>

All values mean ± 1 SD. HD: \( n = 12 \); LD: \( n = 15 \). NS, not significant.
Lower case \( s \) refers to 'short' \( T_2 \) component, lower case \( i \) refers to 'long' \( T_2 \) component.
\(^{*}\) HD: \( n = 5 \); LD: \( n = 6 \).
Metabolite Signal Integrals

In a related study that simulated the characteristics of myelin-associated water (Stanisz et al., 1999), the T2 of myelin-associated water was found independent of myelin content while its signal integral was found to decrease with lower myelin content. This is qualitatively similar to our findings of comparable T2 of the fast relaxing BC components and smaller amplitudes of the fast relaxing BC components in the HD compared with the LD group. This may further support the notion that our 31P MRS findings on BC reflect myelin loss in the HD group. An alternative explanation for the smaller BC integral in HD would be the shift of a small PL fraction into the very rigid PL pool (motion-restricted PL), where it would be invisible to our in vivo 31P MRS measurements at TE = 0.6 msec. This possibility, in addition to our finding of slightly decreased T2, is also consistent with the hypothesis of higher rigidity of PL in HD as a chronic adaptation to the fluidizing effects of acute alcohol.

The smaller BC integral in HD compared with LD taken together with the lack of any correlation with drinking measures suggests (a) that the BC integral is affected by chronic consumption of EtOH over time and/or (b) that it reflects tolerance to or dependence on EtOH. The lack of FH effects on BC suggest that these results do not reflect genetic differences but are the result of differences between groups in alcohol use.

Metabolite Signal Integrals

The integral of the narrow PDE resonances representing GPE and GPC was 5% lower in HD compared with LD. Although not significant, this result is consistent with our previous study (Meyerhoff et al., 1995), which reported an 8% reduction in a much larger cohort of heavy drinkers that was statistically significant. These findings suggest that chronic alcohol abuse is associated with alterations of PL breakdown products that can be observed in vivo.

T2 Relaxation

The T2 relaxation time of the BC is a sensitive indicator of the local mobility of macromolecules. These relaxation measurements showed no change in the T2 value and an insignificantly shorter T2 in HD compared with LD. This trend toward shorter T2 values is consistent with increased rigidity of bilayer membranes in chronic drinkers, demonstrated previously only in isolated membranes (see Introduction). Because our studies provide the first human in vivo measures of the effect of chronic ethanol on the fluidity of PL, no comparison data are available. Previous measurements (Kilby et al., 1990) of the transverse relaxation time of phosphodiesters in human controls performed at 1.9 T reported a single T2 value of around 2 msec for membrane bilayer PL. This value is very close to T2 obtained in our measurements. However, in that study (Kilby et al., 1990), the BC spectrum was not separated from the narrow resonance and the T2 magnetization-decay curve was obtained from peak heights, not integrals, of the broad and narrow resonances superimposed at different TE. With the separation of BC from the raw spectrum and its integration, we were able to observe the multieponential nature of BC and assign both a fast and a slower relaxing component to the BC resonance.

The fast relaxing component with T2 of 1.9 msec may be assigned to fairly mobile PL in membrane bilayers. This is consistent with the literature on T2 relaxation measurements of membranes that used the same T2 fitting algorithm (Kilby et al., 1990; Rajan et al., 1981). The slower relaxing component with T2 of 7 to 8 msec may be from PL in vesicles and mobile bilayers, which are tumbling at a faster rate than planar bilayer structures. These T2 values are somewhat shorter than those reported in the previous study (Kilby et al., 1990), presumably because we separated the BC spectrum from the narrow resonances of the PL breakdown products.

T1 Relaxation

The measured T1 tended to be higher in HD than LD subjects and comparable to previous low-field measurements in human (McNamara et al., 1994) and gerbil brain (Kanashiro et al., 1990). Although T1 measurements were only performed on a small group of participants, the significantly higher T1 values in HD was another indication of the postulated higher rigidity of PL in HD. As mentioned earlier, T1 values for the narrow resonance metabolites were not reliable. This was simply due to the selection of TI to ensure a high S/N ratio for the BC resonance. For this reason, these TI values were not optimal for the longer T1’s of the narrow-line resonances.

Limitations

Because the experiments cannot measure the most rigid PL components, they may not necessarily reflect the characteristics of the entire PL pool in human brain. Nevertheless, the described measurements of the BC signal are sensitive to certain changes of PL in membranes that may be associated with prolonged heavy drinking. Because the BC spectrum is from PL resonances that are homogeneously broadened due to restricted molecular PL mobility, it is impossible to monitor individual phospholipid resonances or to further assign the BC spectrum to myelin or other PL. While proton decoupling would increase the spectral resolution of PL breakdown products, it would not improve spectral separation of individual BC components. The low spatial resolution of localized 31P MRS is another limiting factor that prevents examination of PL in gray and white matter tissues separately. However, because of known effects of chronic alcohol consumption on white matter, we chose to place the VOI in the centrum semi-
ovale to sample primarily white matter. It would be difficult to extend these studies to primarily gray matter volumes because significant reductions of VOI size without increasing measurement time is not a viable option, because of low S/N due to the low intrinsic sensitivity of the $^{31}$P nucleus and fast decay of the BC signal. Regional spectroscopic information on the BC using routine $^{31}$P MR spectroscopic imaging methods (e.g., McNamara et al., 1994) is informative only if the time after signal excitation that is required for phase encoding can be kept significantly shorter than the $T_2$ of the fast relaxing component of the BC signal (Estilaei et al., 2000a). Finally, the accuracy of the measured absolute $T_2$ relaxation time values is constrained by the assumptions made in the chosen $T_2$ fitting model. Therefore, the assignment of the different $T_2$ values to specific PL components needs to be treated with caution. The observed trend of a $T_2$ group difference, however, is unaffected by this limitation.

CONCLUSIONS

This study demonstrates that $^{31}$P MRS provides a powerful and practical approach for measuring in vivo changes of the amount and fluidity of PL in white matter regions of the human brain. Specifically, the smaller, broader component in HD compared with LD suggests altered white matter PL associated with long-term chronic alcohol abuse. However, it remains unclear whether these findings reflect a global brain PL abnormality, a general white matter abnormality, or a more specific myelin abnormality. These findings exist in the absence of supraventricular white matter loss, and predate white matter volume loss commonly observed in elderly chronic heavy drinkers. In vivo $^{31}$P MRS may provide valuable information regarding possible biochemical mechanisms that underly chronic alcohol-induced structural changes detected by CT or MRI. Because these biochemical abnormalities are reflective of probable adaptive mechanisms in response to chronic use of alcohol, this approach can be used also to investigate the degree of potential reversibility of these changes with long-term abstinence (Estilaei et al., 2000b).


