Effects of Abstinence From Alcohol on the Broad Phospholipid Signal in Human Brain: An In Vivo $^{31}$P Magnetic Resonance Spectroscopy Study

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

**Background:** In vivo phosphorus magnetic resonance spectroscopy ($^{31}$P MRS) at a magnetic field strength of 1.5 T allows measurement of fairly mobile membrane phospholipids in the human brain. We previously showed that subjects who are heavy drinkers had a smaller signal and a shorter transverse relaxation time ($T_2$) of white matter phospholipids than light drinkers, which suggested lower concentrations and molecular mobility of phospholipids in heavy drinkers. The purpose of the present study was to measure if such chronic alcohol-induced white matter tissue changes are persistent in long-term abstinent alcoholics.

**Methods:** Fourteen abstinent alcoholics (mean age 45 years, seven men and seven women) were studied by localized $^{31}$P MRS in the centrum semiovale and were compared with 13 male, alcohol-dependent, heavy drinkers and 23 nondependent light drinkers (17 men, 6 women) of similar age. Methods for measurements of the broad membrane phospholipid signal and its relaxation time were described previously.

**Results:** Phospholipid concentrations and relaxation times in alcoholics abstinent for an average of 31 months were not significantly different from those measured in light drinkers. The contribution of fast and slowly relaxing signal components to the broad phospholipid signal, however, was still different in abstinent alcoholics compared with light drinkers. No effects of sex or of family history of alcoholism were noted on any of our spectroscopic measures within the light-drinking or abstinent groups.

**Conclusions:** Most of our results suggest at least partial recovery of chronic alcohol-induced white matter phospholipid damage with long-term abstinence. They offer myelination changes and/or dendritic rearborization as a possible mechanism for the commonly observed white matter volume gain with prolonged abstinence. But the results also suggest a persistent abnormality in the nature and/or physical properties of white matter phospholipids in long-term abstinent alcoholics.

**Key Words:** Magnetic Resonance Spectroscopy, Phospholipid, Abstinence, Myelin, Brain.
possibly with a different central nervous system morbidity than middle-aged to elderly alcoholics typically recruited from treatment facilities and studied shortly after cessation of drinking (Drake et al., 1994; Pfefferbaum et al., 1995; Zipursky et al., 1989). Compared with our male LD sample, white matter and gray matter volumes of the entire brain were not lower and whole-brain cerebral spinal fluid (CSF) volumes were not higher in the active heavy drinkers (Estilaei et al., 2001). In animal brain, the major fraction of the BC resonance originates from myelin (Kanashiro et al., 1990). If this also applies to humans, the lower BC signal in the white matter of the HD group may reflect changes in myelination and/or demyelination that occurred without concomitant magnetic resonance imaging (MRI)-detectable white matter loss. In addition, a trend to shorter relaxation time (T2) of the phospholipids that contribute to the BC resonance in the HD versus LD samples (Estilaei et al., 2001) suggested lower molecular mobility of phospholipids in membranes of the HD group (i.e., higher membrane rigidity), consistent with studies performed in isolated cell membranes exposed chronically to alcohol (Beaugé et al., 1985; Chin and Goldstein, 1977; Foudin et al., 1986; Harris et al., 1984a,b,c; Littleton and John, 1977; Rottenberg et al., 1981; Sun and Sun, 1983). Knowledge of the specific neurobiological processes that underlie structural changes, their interrelationships, and their potential reversal with abstinence is of great significance for understanding brain functional changes associated with alcohol abuse and natural recovery.

The purpose of the present study was to measure the concentration of phospholipids that contribute to the BC signal and their molecular mobility in abstinent alcoholics (AA) who had undergone treatment for their alcohol problems. We hypothesized that phospholipid measures in this long-term AA sample would fall between those in LD and HD samples, which would suggest at least partial reversibility of phospholipid damage with abstinence. Similar or more phospholipid damage in AA subjects than in active HD subjects would suggest greater and/or persistent phospholipid abnormalities in the treated alcoholic than detectable in active HD subjects.

METHODS

Subjects

Fifty subjects were studied according to protocols approved by the local Institutional Review Board and after written informed consent was obtained. Twenty-three subjects were classified as LD (17 men and 6 women; mean age ± 1 SD, 38 ± 10 years; range, 24–60; p = 0.08 vs. HD) with a lifetime average alcohol consumption of 18 ± 16 drinks per month. The male LD sample was described previously (Estilaei et al., 2001). Thirteen were men classified as HD (44 ± 9 years; range, 29–51) with an average alcohol consumption of 185 ± 100 drinks per month (median = 178) over 27.0 ± 7.5 years of use (one HD subject reported a monthly average of 890 drinks; he was not included in the mean). This was the same group described previously (Estilaei et al., 2001). Fourteen were abstinent alcoholics (AA subjects; 7 men and 7 women, 45 ± 6 years; range, 36–57; p = 0.02 vs. LD and p = NS vs. HD) with a monthly average alcohol consumption of 108 ± 64 drinks while drinking (median = 89; p = 0.03 vs. HD). They used alcohol for an average of 29.0 ± 4.7 years (p = NS vs. HD) and, at the time of study, they had been abstinent from alcohol for an average of 31 ± 14 months (median = 30, range, 8–59 months). All AA and HD subjects satisfied DSM-IV criteria for lifetime dependence on alcohol. Lifetime drinking (and other drug use) history was obtained by using standard methods (Skinner and Sheu, 1982; Sobell and Sobell, 1994) where one drink was considered an alcoholic beverage that contained 11 to 13 g of alcohol. All subjects were recruited from the community via advertisements. Thus, the HD subjects were non-treatment-seeking individuals, whereas the AA subjects had participated successfully in alcoholism treatment programs. The HD and AA samples did not differ in age, drinking duration, total quantity of alcohol consumed over their lifetime, quantity of nonpeak use, or monthly average peak consumption. HD subjects tended to have a longer alcohol peak use duration than AA subjects (9.1 ± 8.8 years vs. 3.7 ± 5.1 years, p = 0.06) and a greater monthly average alcohol consumption over lifetime (p = 0.03). These differences, however, disappeared when only male subjects were compared in both groups (139 ± 66 drinks/month in male AA subjects vs. 185 ± 100 drinks/month in male HD subjects), consistent with trends for lower consumption in the female versus male AA subjects participating in this study.

Subjects were screened to exclude individuals with a history of substance dependence (other than alcohol); medical, neurological, or psychiatric disorders, including a history of head injury with loss of consciousness; stroke; cerebral infarctions; or other major brain abnormalities on MRI scans. Family history of alcohol use was assessed with a self-administered questionnaire in all but six subjects, three of whom were adopted and did not know their family history. Subjects with a father and/or mother identified as a problem drinker were considered family history-positive (FHP; n = 21, age 43.5 ± 7.0 years). Subjects with only siblings and/or second-degree relatives identified as problem drinkers were considered family history-negative (FHN; n = 23, age 40.2 ± 10.9, p = NS vs. FHP). Mothers of one LD, two HD, and four AA subjects were problem drinkers. The LD sample had a significantly lower family history density than either the HD or AA sample (x2 = 7.08, p < 0.004): Only 4 of the 20 LD subjects were FHP (20%), 9 of the 12 HD subjects were FHP (75%), and 8 of the 12 AA subjects were FHP (67%). Subjects had a light lunch 4 to 6 hr before the study and had a breath alcohol concentration of <0.003% at the time of study. This is the detection limit of the hand-held breathalyzer (Alco-SensorIV, Intoximeters Inc., St. Louis, MO) that was used to test breath samples for alcohol.

MR Measurements

All experiments were performed on a whole-body 1.5 T Magnetom Vision system (Siemens Inc., Iselin, NJ) equipped with a homogeneous double-tuned (31P/1H) birdcage coil that operated at 25.8 MHz for 31P (Matson et al., 1999). Data acquisition and processing methods were identical to our previous studies (see Estilaei et al., 2001 for more details), except that all integral measures were referenced to the same external standard present in the head coil for each study. The standard contained hexamethyldiphosphoroustrichloramide (HMPT), which resonates approximately 120 ppm downfield from the brain spectrum. A vacuum-molded head holder (Vac-Pac, Olympic Medical, Seattle, WA) was used to minimize motion of the subject’s head. The MRI protocols consisted of sagittal T1-weighted localizer scans and axial turbo spin-echo scans acquired along a line that connected the anterior and posterior commissures. The MR parameters such as repetition time (TR) and echo time (TE) were as previously described (TR/TE/T2 = 7000/14/80 msec) (Estilaei et al., 2001). These images were used to place the volume of interest (VOI) in the centrum semiovale and to avoid inclusion of ventricles in the VOI. The VOI contained predominantly white matter. Figure 1 shows the typical location of the fixed VOI (90° 13·22 X 120·109 mm3) and the mean signal and their molecular mobility in abstinent alcoholics (Estilaei et al., 2001). In animal brain, the major fraction of the BC resonance originates from myelin (Kanashiro et al., 1990). If this also applies to humans, the lower BC signal in the white matter of the HD group may reflect changes in myelination and/or demyelination that occurred without concomitant magnetic resonance imaging (MRI)-detectable white matter loss. In addition, a trend to shorter relaxation time (T2) of the phospholipids that contribute to the BC resonance in the HD versus LD samples (Estilaei et al., 2001) suggested lower molecular mobility of phospholipids in membranes of the HD group (i.e., higher membrane rigidity), consistent with studies performed in isolated cell membranes exposed chronically to alcohol (Beaugé et al., 1985; Chin and Goldstein, 1977; Foudin et al., 1986; Harris et al., 1984a,b,c; Littleton and John, 1977; Rottenberg et al., 1981; Sun and Sun, 1983). Knowledge of the specific neurobiological processes that underlie structural changes, their interrelationships, and their potential reversal with abstinence is of great significance for understanding brain functional changes associated with alcohol abuse and natural recovery.

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were obtained with a fixed TR previously (Estilaei et al., 2001; Kilby et al., 1990). This approach allowed for each subject, the average $T_1$ value obtained for a group was used to determine the relative magnetization and $T_1$ for a fast and slowly relaxing $T_2$ component. $T_1$ values measured for each subject were used to correct each individual's $BC$ for $T_1$ effects. Due to low signal to noise, $T_1$ analysis was not carried out for the narrow metabolite resonances.

**RESULTS**

**Broad Component Integral**

Figure 1a shows a representative phosphorous spectrum obtained at TE = 0.6 msec. Applying convolution difference and subtracting the spectra from each other yields a spectrum of the BC (Fig. 1c). Table 1 lists the mean BC (column 1) and metabolite integrals (columns 4–8) without relaxation corrections at TE = 0.6 msec measured for LD, HD, and AA groups. We previously reported on BC differences between male LD and male HD subjects (Estilaei et al., 2001). Although the results reported here include women in the LD sample, they are essentially unchanged from those previous findings. Briefly, the BC integral was 14% smaller in HD subjects relative to LD subjects ($p = 0.0006; 41.5\%$ of BC variance accounted for by group membership). By contrast, no significant differences were observed for any of the metabolite integrals. This latter observation and our previous volumetric analysis of male subjects in both groups (Estilaei et al., 2001) suggest that there were no tissue volume or CSP differences between HD and LD subjects in the whole brain and in the region of interest for $31^P$ MRS. Table 1 also lists the BC integral after both $T_2$ as well as $T_1$ and $T_2$ relaxation corrections. The BC group differences between LD and HD groups remained highly significant ($p < 0.005; >32.2\%$ of BC variance accounted for by group membership). Separate statistical analyses showed no significant BC differences between male and female LD subjects ($p > 0.43; <3.4\%$ of variance of any of the BC measures accounted for by sex). Therefore, male and female LD subjects were pooled in all analyses.

**Effects of Abstinence.** None of the BC measures were different between AA and LD groups. Relative to HD, the uncorrected BC integral in AA was 12% larger ($p = 0.02$). This difference was still significant after $T_2$ relaxation correction ($p = 0.04$) and became a trend after additional correction for $T_1$ relaxation ($p = 0.10$). Although the female AA group showed trends to less severe drinking history measures, BC measures were not different between male and female AA subjects. Figure 2 illustrates the distribution of the BC integral in all three-study groups by family history and sex.

An ANOVA with age as covariate showed that the three groups differed on the raw [$F(2,46) = 6.33, p = 0.004$], the $T_2$-corrected [$F(2,43) = 5.63, p = 0.007$], and the $T_1$-corrected BC integrals.

**Statistical Analysis**

Statistical analyses were performed with the "proc GLM" routine in SAS software (SAS Institute Inc., Cary, NC) with SPLUS (MathSoft, Inc., Cambridge, MA). Groups were compared by using $\chi^2$ tests for categorical data and ANOVA for continuous measures. Comparisons of outcome measures used two-tailed tests with age as covariate. In addition, all three groups were compared by ANOVA and analysis of covariance, with post hoc Tukey tests. Correlations among MR measures, age, or alcohol history measures were assessed by Pearson correlations. Data are reported as mean ± 1 SD, and results were considered significant at the 0.05 level.
not study women (Estilaei et al., 2001). Therefore, the Relaxation Times and BC Integral Components varied for age are shown in Table 2. Distribution of the BC integral in light drinkers (LD), abstinent alcoholics (AA), and heavy drinkers (HD) by family history and sex. Circles, subjects with negative family histories (FHN); triangles, subjects with positive family histories (FHP); solid symbols, males; open symbols, females.

Table 1. Integral of BC and Metabolites With and Without Relaxation Corrections (in Arbitrary Units)

<table>
<thead>
<tr>
<th></th>
<th>BC</th>
<th>BC T&lt;sub&gt;2&lt;/sub&gt; corrected</th>
<th>BC T&lt;sub&gt;1&lt;/sub&gt; &amp; T&lt;sub&gt;2&lt;/sub&gt; corrected</th>
<th>PDE</th>
<th>PME</th>
<th>Pi</th>
<th>PCr</th>
<th>γ-ATP</th>
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<tr>
<td>LD (n = 23)</td>
<td>81.1 ± 6.8</td>
<td>97.5 ± 8.7</td>
<td>108.0 ± 9.6</td>
<td>11.7 ± 1.8</td>
<td>3.1 ± 0.7</td>
<td>2.6 ± 0.6</td>
<td>4.0 ± 0.5</td>
<td>4.7 ± 1.1</td>
</tr>
<tr>
<td>AA (n = 14)</td>
<td>78.0 ± 9.0</td>
<td>92.4 ± 11.8</td>
<td>102.3 ± 13.1</td>
<td>11.1 ± 1.3</td>
<td>2.9 ± 1.2</td>
<td>2.4 ± 0.6</td>
<td>4.2 ± 0.4</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>HD (n = 13)</td>
<td>69.7 ± 10.3</td>
<td>82.9 ± 12.3</td>
<td>93.8 ± 13.9</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>4.2 ± 0.9</td>
</tr>
<tr>
<td>p (LD vs. HD)*</td>
<td>0.0006</td>
<td>0.001</td>
<td>0.004</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>p (LD vs. AA)*</td>
<td>0.02</td>
<td>0.04</td>
<td>0.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>p (LD vs. AA)</td>
<td>NS</td>
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</tbody>
</table>

BC, broad component; PDE, phosphodiester; PME, phosphomonoester; Pi, inorganic phosphate; PCr, phosphocreatine; ATP, adenosine triphosphate; LD, light drinkers; AA, abstinent alcoholics; HD, heavy drinkers.

* Significance p essentially unchanged when comparing male samples only.

T<sub>2</sub> corrected BC integrals [F(2,43) = 4.04, p = 0.02]. Follow-up Tukey tests showed that this was due to significantly smaller BC measures in the HD versus LD group, whereas none of the BC measures was significantly different between LD and AA groups.

Relaxation Times and BC Integral Components

T<sub>1</sub> measurements were performed to obtain information on the rigidity of phospholipids and to apply relaxation corrections to the raw BC integral. The T<sub>2</sub> relaxation curves could not be fit to a monoexponential equation, and a biexponential curve represented the best fit to the experimental T<sub>2</sub> data. Results for all three groups after we covaried for age are shown in Table 2. Including women in the LD sample and covarying for age ameliorated the T<sub>2</sub> differences between LD and HD groups reported earlier (Estilaei et al., 2001). Nevertheless, the T<sub>2</sub> of the slowly relaxing component (T<sub>2s</sub>) was 7% shorter in HD compared with LD, but this difference was not significant. The T<sub>2</sub> of the fast decaying component (T<sub>2f</sub>) and the amplitude of the slowly decaying component (S<sub>e</sub>) were similar in LD and HD groups, as reported earlier. The amplitude of the fast decaying component (S<sub>f</sub>) was 21% smaller in HD relative to LD (p = 0.006), consistent with our previous report that did not study women (Estilaei et al., 2001). Therefore, the amplitude difference of the fast decaying component accounted primarily for the BC integral difference between LD and HD groups. A separate statistical analysis of the T<sub>2</sub> data from LD subjects revealed no sex effects (p > 0.38; <3.9% of variance for any of the T<sub>2</sub> and S measures accounted for by sex).

Effects of Abstinence. The T<sub>2</sub> value of the fast decaying component (T<sub>2f</sub>) and that of the slowly decaying component (T<sub>2s</sub>) were similar for all three groups. The amplitudes of the slowly decaying component (S<sub>e</sub>) were similar in all three groups, with a trend to a larger S<sub>e</sub> component in AA compared with HD subjects (p = 0.08). The amplitude of the fast decaying component (S<sub>f</sub>) was similar in AA and HD groups. The S<sub>f</sub> component, however, was 17% smaller in AA than in LD subjects (p < 0.02). This was in contrast to the total BC integral (sum of S<sub>e</sub> and S<sub>f</sub>) described previously, which was similar in LD and AA subjects. No significant sex effects were observed on T<sub>2</sub> and S<sub>e</sub> measures in the AA sample (p > 0.29; <9.3% of variance accounted for by sex), but S<sub>f</sub> tended to be larger in male versus female AA subjects (45.3 ± 11.0 vs. 38.1 ± 8.4; p = 0.19).

Analysis of covariance indicated that the groups differed on the amplitudes of the fast relaxing components (S<sub>f</sub>) [F(2,43) = 4.96, p = 0.01], due to significantly smaller S<sub>f</sub> in HD subjects compared with LD subjects, but no significant AA versus LD difference was observed.

T<sub>1</sub> Relaxation. T<sub>1</sub> relaxation times were measured in all groups to correct BC integrals for T<sub>1</sub> relaxation effects (Table 2). The average age-corrected T<sub>1</sub> in HD subjects tended to be 8% longer (p = 0.08) than in the LD group. The longer T<sub>1</sub> in HD subjects accounted for about 2% decrease in the overall BC integral in HD. The average BC T<sub>1</sub> in AA subjects was very similar to that of LD and insignificantly smaller than in HD.

Associations Between Drinking History and Spectroscopic Measures

The BC integrals within HD and AA groups were not correlated with any of the measures of drinking history. However, within the AA group, there were positive linear correlations between the length of abstinence and both T<sub>2</sub> relaxation times (T<sub>2f</sub>; r = 0.76, p = 0.002, see Fig. 3, and T<sub>2s</sub>; r = 0.62, p = 0.02). This suggests recovery to normal T<sub>2</sub> values with extended abstinence. The strengths of the correlations were similar for female (r = 0.85, p = 0.03) and
male AA subjects \((r = 0.73, p = 0.10)\). Age was not correlated significantly with BC or \(T_2\) measures in either group, except for a significant negative correlation of age with \(T_{2a}\) within the LD group \((r = -0.53, p = 0.01)\).

FHP and FHN groups did not differ in any of the BC or relaxation time measures, regardless of whether we analyzed all subjects or AA and HD subjects combined.

**DISCUSSION**

These in vivo \(^{31}\)P MRS studies extend our previous findings (Estilaei et al., 2001) by including women in the LD cohort and by studying both male and female abstinent alcoholics. No sex effects were noted on any of our spectroscopic measures within the control or abstinent groups. The results show that most of our MR spectroscopic phospholipid measures in alcoholics abstinent for 31 months are generally closer to those of LD subjects than to those of active HD subjects. This suggests at least partial recovery of chronic alcohol-induced white matter phospholipid damage with long-term abstinence.

Our active drinking population differs from the alcoholics typically studied, in that most other studies on brain effects of chronic alcohol abuse involved individuals from alcoholism treatment centers at several weeks after cessation of drinking (i.e., "recently detoxified alcoholics"). Recent studies, however, suggest that chronic alcohol-induced damage of brain structure and metabolism, cognition, and blood flow improve at least partially in just a few weeks of abstinence. Therefore, by studying active heavy drinkers from the community soon after their last drink (i.e., those who have not experienced treatment-associated abstinence sufficiently long for short-term recovery), we may get a better picture of the full extent of brain damage due to chronic heavy drinking. On the other hand, however, one might argue that active HD subjects do not display the same degree of brain damage typically observed in the treatment-seeking alcoholic, especially if primary brain damage is thought to be mediated more by withdrawal than alcohol use per se. If this is indeed so, then the differences in BC integrals, when we compare HD and AA groups, would be smaller than between recently detoxified alcoholics and long-term abstainers and would underestimate the degree of recovery in our AA subjects.

**Broad Component Integral and Structural Neuroimaging**

Neuroimaging studies of chronic drinkers in alcoholism treatment programs show widespread volume deficits in gray, subcortical gray, and white matter (Carlen et al., 1986; Jernigan et al., 1982, 1986, 1991; Pfefferbaum et al., 1992, 1993; Shear et al., 1994; Sullivan et al., 1996), often accompanied by increases of CSF spaces. Correlations between these tissue volume losses and drinking severity have not been demonstrated consistently (Shear et al., 1994; Pfefferbaum et al., 1995; Trabert et al., 1995). Postmortem studies suggest that cerebral white matter is more vulnerable to the neurotoxic effects of chronic alcohol consumption than gray matter (Harper and Kril, 1990; Wiggins et al., 1988), and heavy alcohol consumption has been shown to be associated with an accelerated age-related myelin loss (Wiggins et al., 1988). Chronic alcohol-induced brain shrinkage has been related to white matter loss (Harper et al., 1985), the amount of which, in particular in frontal brain, has been associated with maximum daily alcohol consumption (Kril et al., 1997). Although our volumetric studies of currently active, relatively young HD subjects did not show white matter tissue volume loss in the whole brain or in the MRS region (but their dorsolateral gray matter

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**Table 2. Relaxation Times \((T_1, T_2)\) (in msec) and Relative Magnetization \(S\) for BC Components (in arbitrary units)**

<table>
<thead>
<tr>
<th></th>
<th>(S_a)</th>
<th>(S_b)</th>
<th>(T_{2a})</th>
<th>(T_{2b})</th>
<th>(T_1^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD ((n = 21))</td>
<td>59.7 ± 9.5</td>
<td>37.8 ± 6.5</td>
<td>1.8 ± 0.2</td>
<td>7.6 ± 1.2</td>
<td>859 ± 81</td>
</tr>
<tr>
<td>AA ((n = 14))</td>
<td>50.7 ± 9.3</td>
<td>41.7 ± 10.2</td>
<td>1.8 ± 0.4</td>
<td>6.7 ± 1.2</td>
<td>856 ± 158</td>
</tr>
<tr>
<td>HD ((n = 12))</td>
<td>47.3 ± 11.7</td>
<td>35.6 ± 5.3</td>
<td>1.9 ± 0.3</td>
<td>7.1 ± 0.9</td>
<td>932 ± 84</td>
</tr>
</tbody>
</table>

Lowercase \(s\), "short" \(T_2\) component; lowercase \(l\), "long" \(T_2\) component; \(S_a\), amplitude of the slowly relaxing component; \(S_b\), amplitude of the fast relaxing component; LD, light drinkers; AA, abstinent alcoholics; HD, heavy drinkers.

* HD, \(n = 5\); LD, \(n = 6\); AA, \(n = 4\); \(^{\text{**}}\) significance \(p\) essentially unchanged when comparing male samples only; \(^{\text{a}}\) differences disappear when comparing female samples only; for \(S_p = 0.06\) when comparing male samples only.

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**Fig. 3. Correlation between length of abstinence and \(T_2\) relaxation time of the slowly relaxing BC component \((T_{2b})\) in male (squares) and female (triangles) abstinent alcoholics. The regression line represents a linear fit to data from all subjects. The regression coefficient is \(r = 0.76\) with \(p = 0.002\).**

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**Note:**

- Although our volumetric studies of currently active, relatively young HD subjects did not show white matter tissue volume loss in the whole brain or in the MRS region (but their dorsolateral gray matter...
brain regeneration, and it indicates a potential biochemical hypothesis that brain volume changes in alcoholics may effects of alcohol and the reversibility of these effects with frequent discussion). Successful abstinence from alcohol (however, see subsequent discussion).

is consistent with remyelination and possibly regrowth of white matter phospholipids. These 31P MRS studies are important because they detect significant damage to white matter phospholipids in active HD that may predate the white matter volume loss typically observed in neuroimaging studies of recently detoxified alcoholics. It is unclear how these phospholipid changes may be associated with recently demonstrated white matter microstructural abnormalities in detoxified alcoholic men (Pfefferbaum et al., 2000), but they may be linked through myelination changes.

Effects of Abstinence. Neuroimaging studies in successfully abstinent alcoholics suggest that chronic alcohol-induced morphological abnormalities recover at least partially with duration of abstinence (Artmann et al., 1981; Carlen et al., 1978, 1984; Drake et al., 1994; Muuronen et al., 1989; Pfefferbaum et al., 1995; Ron et al., 1982; Shear et al., 1994; Trabert et al., 1995; Zipursky et al., 1989). An early computed tomography study (Carlen et al., 1978) provided evidence of partial reversal of cortical, sulcal, and ventricular dilation in chronic drinkers abstinent for almost a year. Decreases in ventricular volume (Zipursky et al., 1989) and increases in brain tissue density (Trabert et al., 1995) have been demonstrated within less than a month of abstinence, whereas other studies reported white matter and gray matter volume increases together with ventricular volume decreases within several months to 1 year of abstinence (Drake et al., 1994; Pfefferbaum et al., 1995; Shear et al., 1994; Zipursky et al., 1989). These tissue volume increases in the brain of recovering alcoholics have been associated with remyelination and regrowth of axonal/dendritic arbor damaged from chronic heavy drinking (Carlen et al., 1984; Dlugos and Pentney, 1997; Durand et al., 1989; Harper and Kril, 1990; Pfefferbaum et al., 1995; Shear et al., 1994). In this study, we found that the white matter BC integrals in long-term AA subjects are similar in magnitude to those in LD subjects, which suggests similar phospholipid concentrations in white matter and, thus, recovery from low phospholipid concentrations in HD subjects. This is consistent with remyelination and possibly regrowth of axonal/dendritic arbor associated with phospholipid membrane regeneration, and it indicates a potential biochemical mechanism responsible for partial volumetric recovery with successful abstinence from alcohol (however, see subsequent discussion).

The specific mechanisms that mediate the neurotoxic effects of alcohol and the reversibility of these effects with abstinence are poorly understood. Carlen et al. (1984) hypothesized that brain volume changes in alcoholics may reflect two processes: (a) irreversible atrophy as a result of neuronal loss, and (b) atrophy that is reversible due to reformation of dendritic arbor and perhaps original neuronal body size, which leads to increased tissue density with abstinence as observed by computed tomography (Trabert et al., 1995). The rearborization hypothesis obtained support from animal studies (Dlugos and Pentney, 1997; Durand et al., 1989) and was formulated into a general model of alcohol-induced damage and recovery with abstinence by Harper and Kril (1990). The BC changes observed in chronic alcohol abuse and with successful recovery may reflect these processes, and they may be associated with or may occur together with remyelination.

Within the HD and AA groups, no correlation was found between the BC integral and measures of drinking severity. Therefore, when we eliminated those HD subjects from analysis who contributed most to the significant difference in monthly average alcohol consumption between HD and AA subjects, or when we compared only male HD and AA subjects, the BC group differences remained largely unchanged. In addition, the somewhat lower monthly average alcohol consumption in female (76 ± 46 drinks/month) vs. male AA subjects (139 ± 66 drinks/month, p = 0.06) did not predict a higher BC integral in women. This may suggest a greater vulnerability or slower recovery of the female brain to white matter phospholipid damage. Similar to our failure to demonstrate an association between drinking severity and degree of phospholipid damage in active and abstinent drinkers, volumetric studies have generally failed to show an association between drinking severity and volume loss in recently detoxified alcoholics (Pfefferbaum et al., 1995; Shear et al., 1994; Trabert et al., 1995).

Relaxation Times and Components of Broad Component Integral

The molecular mobility of phospholipids was probed by measuring T2 relaxation times. In contrast to our previous study in male subjects that suggested higher rigidity of phospholipid membranes in chronic HD, age-corrected T2 measures in this larger LD sample, which included women, were not significantly smaller than in HD. Thus, these measurements did not suggest markedly greater membrane rigidity due to chronic heavy drinking. Nevertheless, in AAs, we observed a significant association between length of abstinence and T2 of the slowly relaxing BC component, which suggested at least some fluidization of membranes during abstinence and reflected reversible processes of chronic alcohol-induced membrane alterations.

Our relaxation time studies also show that, although the T2 relaxation times of the BC components and the total BC integral were similar in AA and LD subjects, the fast relaxing BC component S2 was smaller in AA than in LD. This suggests a difference in the nature and/or physical properties of white matter phospholipids in AAs rather than a simple reversibility to control conditions. It may reflect a persistent phospholipid abnormality in the white matter of alcoholics abstinent for 31 months and may signal

volumes were lower) (Estilaei et al., 2001; Goldmann et al., 1999), our 31P MRS studies in the same subjects and the present 31P MRS findings in a similar subject cohort found lower white matter phospholipid concentrations in HD than LD subjects. These findings are consistent with chronic alcohol-induced white matter changes, including possible demyelination and/or changes in the composition of white matter phospholipids. These 31P MRS studies are important because they detect significant damage to white matter phospholipids in active HD that may predate the white matter volume loss typically observed in neuroimaging studies of recently detoxified alcoholics. It is unclear how these phospholipid changes may be associated with recently demonstrated white matter microstructural abnormalities in detoxified alcoholic men (Pfefferbaum et al., 2000), but they may be linked through myelination changes.

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that white matter in these subjects, although "normal" by one measure, may not be fully recovered or equivalent to white matter in light-drinking controls.

T2 relaxation time values were similar in LD and AA subjects and tended to be longer in HD compared with LD subjects. This also suggests a normalization of T2 relaxation time with abstinence, adding further evidence for some reversibility of white matter damage and/or changes in the composition of phospholipids during prolonged abstinence.

Study Limitations

Sex differences were not detected on our measures of the BC integral and relaxation times in LD and AA subjects. However, only large differences would have been detectable given our relatively small sample size, and further studies of sex effects need to involve more subjects and an equal sex distribution within the samples.

Because of experimental limitations regarding the shortest possible echo time in our ISIS experiments, our measurements do not capture the most rigid phospholipid components with very short T2. Therefore, they do not necessarily reflect the characteristics of the entire phospholipid pool in the brain. Nevertheless, the described measurements of the BC signal are sensitive to certain changes of phospholipids in membranes that may be associated with prolonged heavy drinking and recovery thereof. Improvements in experimental design and pulse characteristics will allow measurement of more rigid components in the future.

The low spatial resolution of localized 31P MRS is another limiting factor. It prevents examination of small, localized regions in brain membranes or even of regional phospholipid damage 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 semiionale to primarily sample white matter. It would be difficult to extend these studies to primarily gray matter volumes due to signal-to-noise limitations connected with the low intrinsic sensitivity of the 31P nucleus and fast decay of the BC signal. Regional spectroscopic information on the BC that uses routine 31P MR spectroscopic imaging methods (e.g., Murphy-Boesch et al., 1993) is informative only if the time after signal excitation that is required for phase encoding can be kept significantly shorter than the T2 of the fast relaxing component of the BC signal (Estilaei et al., 2000b).

The tissue and CSF composition of the 31P MRS region was not computed in the AA subjects. In our previous report on LD and HD subjects, however, we found no group differences of these measures (Estilaei et al., 2001). Furthermore, the metabolite integrals in the MR spectra of AA subjects were virtually identical to those of LD and HD subjects, which would not have been the case if different amounts of CSF were contributing to the MRS region. Finally, our AA subjects had been abstinent for an average of 31 months, allowing for significant recovery of any tissue loss that might have been present after cessation of drinking. Therefore, it is reasonable to assume that the tissue content of the MRS VOI in the AA group was equivalent to that in the LD and HD groups.

Finally, our results are cross-sectional, they reflect subject variability, and they were obtained in actively drinking subjects and abstinent alcoholics who, despite similar drinking history measures, were likely not matched to the active drinkers on their neuropathological damage at the onset of recovery. These limitations can be overcome only in longitudinal studies of recovering alcoholics. Despite these limitations, our in vivo 31P MRS findings are consistent with other neuroimaging and pathologic studies that report white matter changes with chronic alcohol use and prolonged abstinence. They contribute unique information about possible mechanisms that underlie alcohol-induced brain damage and recovery thereof. Further studies are needed that assess the functional/behavioral significance of the detected white matter damage and its potential reversibility. More generally, in vivo 31P MRS appears promising for measuring longitudinal brain phospholipid changes in the investigation of diseases associated with membrane damage.

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