Quantitative hepatic phosphorus-31 magnetic resonance spectroscopy in compensated and decompensated cirrhosis


Quantitative hepatic phosphorus-31 magnetic resonance spectroscopy in compensated and decompensated cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 287: G379–G384, 2004. First published February 5, 2004; 10.1152/ajpgi.00418.2003.—Few studies have examined the physiological/biochemical status of hepatocytes in patients with compensated and decompensated cirrhosis in situ. Phosphorus-31 magnetic resonance spectroscopy (31P MRS) is a noninvasive technique that permits direct assessments of tissue bioenergetics and phospholipid metabolism. Quantitative 31P MRS was employed to document differences in the hepatic metabolite concentrations among patients with compensated and decompensated cirrhosis as well as healthy controls. All MRS examinations were performed on a 1.5-T General Electric Signa whole body scanner. The concentration of hepatic phosphorylated metabolites among patients with compensated cirrhosis (n = 7) was similar to that among healthy controls (n = 8). However, patients with decompensated cirrhosis (n = 6) had significantly lower levels of hepatic ATP compared with patients with compensated cirrhosis and healthy controls (P < 0.02 and P < 0.009, respectively) and a higher phosphomonooesther/phosphodiester ratio than controls (P < 0.003). The results of this study indicate that metabolic disturbances in hepatic energy and phospholipid metabolism exist in patients with decompensated cirrhosis that are not present in patients with compensated cirrhosis or healthy controls. These findings provide new insights into the pathophysiology of hepatic decompensation.

CIRRHOSIS AND ITS ASSOCIATED complications are a major cause of morbidity and mortality worldwide (35). The clinical spectrum of cirrhosis is broad, including patients who are asymptomatic and unaware of their condition as well as those who require hospitalization for life-threatening emergencies (5, 46). Between these extremes is an insidious clinical course in which compensated cirrhosis progresses to a decompensated state. During the early stages of cirrhosis the large functional reserve of the liver is able to compensate for changes in tissue architecture brought about by bridging fibrosis and abnormal nodular formation. Hence, patients may only experience nonspecific symptoms such as fatigue, weakness, and general malaise. Eventually the disease progresses to the point at which the hepatic reserve is unable to compensate for hepatocyte loss and structural distortions in the liver. At this late stage, signs of hepatic dysfunction ensue, including jaundice, ascites, variceal hemorrhage, and/or portal systemic encephalopathy. The development of these complications marks the transition from compensated to decompensated cirrhosis and affords the patient a poor prognosis.

The clinical features and gross pathophysiology surrounding hepatic decompensation are well described (5, 46); however, little is known regarding cellular events in end-stage liver disease. Functionally, in decompensated cirrhosis the synthetic and excretory capacity of the liver is compromised, because low levels of albumin, prolonged clotting times [international normalized ratio (INR)], and high levels of bilirubin are commonly documented (18). Several quantitative liver function tests convey a similar message in that the decompensated cirrhotic liver has a reduced capacity to metabolize/detoxify exogenous compounds (13). To date, few studies have attempted to document the cellular events that exist in compensated and decompensated cirrhosis, largely because most methods require invasive tissue sampling, which is generally avoided in this group of ill patients with coagulopathies. One technique that noninvasively provides valuable metabolic information regarding tissues in situ is phosphorus-31 magnetic resonance spectroscopy (31P MRS) (8, 12, 22, 24, 29, 44). 31P MRS is a radiological technique that permits the simultaneous detection and quantitation of several cytosolic phosphorus-containing compounds involved in energy metabolism (ATP and P3) and membrane phospholipid metabolism [phosphomonoesters (PME) and phosphodiesters (PDE)]. To date, several studies have used in vivo MRS to study hepatic metabolic changes during the progression of chronic liver disease (16, 20, 25, 31, 43). These studies commonly report increases in hepatic PTE relative to ATP or PDE; however, little is known regarding the level of individual metabolites and the significance of changes therein during hepatic decompensation. Thus in the present study we employed quantitative 31P MRS to document and compare the hepatic metabolite profile of patients with compensated and decompensated cirrhosis.

MATERIALS AND METHODS

Subjects. The study population consisted of 17 chronic hepatitis C patients. Their mean age was 51 years (range 28–73 years), and 13 were male. The presence of cirrhosis was documented histologically and/or by computed tomography or ultrasound features of cirrhosis, i.e., irregular, nodular liver surfaces with accompanying signs of portal hypertension such as the presence of ascites, esophageal varices, and/or splenomegaly. Patients were classified as having either compensated or decompensated cirrhosis on the basis of clinical and radiological findings. Decompensated cirrhosis was defined as the presence of at least two of the following five criteria: ascites, hyperbilirubinemia, peripheral edema of noncardiac or renal origin, hy-

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poalbuminemia, and an INR > 1.3. Nine patients with compensated cirrhosis and eight with decompensated cirrhosis were studied. Eight volunteers (6 males, 2 females, mean age 51 years, range 41–60 years) with no history or evidence of liver disease served as healthy controls.

**Magnetic resonance examination.** Before each magnetic resonance (MR) scan, subjects were required to fast for 4 h. MR examinations were performed on a 1.5-T General Electric Sigma whole body MR system. Subjects were positioned supine within the scanner, and a 15-cm transmit/receive phosphorus surface coil, tuned and matched to 25.85 MHz, was placed directly above the subject’s liver. A small vial containing 0.5 M phenylphosphonic acid (PPA) was placed at the center of the coil to assist with subject positioning during proton imaging, calibration of the radio frequency (RF) field strength at the region of interest (ROI), and quantitation of metabolite concentrations. Proton signal was used for shimming, and localizer images in the coronal and axial planes were then obtained with the body coil to ensure correct coil positioning. Further automated and manual localized shimming on the ROI within the liver was performed by using a STEAM sequence with a typical voxel size of 70 × 70 × 40 mm (lateral, vertical, and axial dimensions, respectively). For 31P MRS at 25.85 MHz, the transmit power to obtain a 90° flip angle was determined for the PPA reference vial at the center of the coil by using a 900-μs slice selective gaussian RF pulse. A fully relaxed spectrum (relaxation time T1 = 7 s) of the entire 4-cm axial slice was acquired, and the area of the PPA peak at 14.3 ppm was used for measurements of coil loading. On the basis of the characteristics of the B1 field of the surface coil, the transmit power for a 45° flip angle was determined at the center of the ROI (typically at a depth of 7 cm) for subsequent localized MRS. Localized 31P liver spectra were acquired by using two-dimensional chemical shift imaging (2D-CSI). Data acquisition parameters were as follows: field of view = 48 cm (horizontal) × 48 cm (vertical), 8 averages, relaxation time T1 = 1.5 s, matrix size = 12 × 12, acquisition size 1,024 pt, zero-filled to 2,048 pt, and sweep width 2,500 Hz.

**Data processing.** Data processing was accomplished by using SAGE software (General Electric). Briefly, the free induction decay underwent 10-Hz exponential line broadening before Fourier transformation and phase corrections were performed on the resulting spectra. Initial first-order phase values were corrected by 2π radians for each dwell period during which data collection was delayed (1). The phase was then further improved with minor adjustments to zero and first-order phase values. Thereafter, cubic spline baseline correction was performed by a consistent selection of 8–10 baseline points on the basis of prior knowledge of the well-characterized inverse sinc-like baseline roll produced in in vivo CSI spectra (41). Necessary adjustments were made if the prescribed baseline points occurred on noise spikes or troughs. Peaks were registered relative to α-ATP resonance (~10 ppm), which served as an internal chemical shift reference (6). Finally, spectra were analyzed with a frequency domain-fitting function where peak integrals were calculated with gaussian curves. The PME, P, PDE, and phosphocreatine (PCr) (when necessary) signals were treated as singlets, γ- and α-ATP peaks as doublets, and the β-ATP resonance as a triplet. Corrections for minor contributions of metabolite signal arising from overlying skeletal muscle were performed. Based on the percentage of PCr in the liver spectra, relative amounts of muscle signal contributing to each metabolite were calculated according to previously published data (3, 21). These values were then subtracted from the appropriate integral to give a “pure” liver reading.

**Quantitation.** For quantitation of hepatic metabolites, simulated phantom experiments were performed as described by Meyerhoff et al. (28). A 20-liter plastic carboy containing 50 mM sodium phosphate served as a phantom on which identical MR examinations were performed regularly throughout the experiment. The various metabolite concentrations were determined by the equation

\[ C = C_p \times I/I_p \times N_p/N \times S_p/S \times I_{ref}/I_{ref} \]

where C is absolute metabolite concentration in mmol/l, Cp is concentration of phantom solution used for calibration in mmol/l, I/Ip is corresponding signal integrals, Np/N is corresponding number of signal averages, S/Sp is corresponding saturation factors calculated from measured T1 times, and Iref/Iref is corresponding signal integral of reference sample. Literature T1 values reported for liver were used in the calculation for saturation factors (10).

Hepatic ATP concentration was based on the peak area of the β-ATP resonance, which is free of other phosphorylated adenosine species (14).

**Liver function.** Liver enzyme and function tests were performed by the Clinical Chemistry Laboratory at the Health Sciences Centre (Winnipeg, MB, Canada) within 1 wk of the date of the MR examination. Child-Pugh’s scores were assigned as follows: 1 point each for no encephalopathy, no ascites, bilirubin < 20 μmol/l, albumin > 35 g/l, and INR < 1.7; two points each for grade 1 or 2 encephalopathy, minimal-to-moderate ascites, bilirubin 40–60 μmol/l, albumin 28–35 g/l, and INR 1.7–2.2; and three points each for grade 3 or 4 encephalopathy, severe ascites, bilirubin > 60 μmol/l, albumin < 28 g/l, and INR > 2.2. Subjects with class A cirrhosis were defined as those with points totalling 5–6; class B, 7–9 points; and class C, 10–15 points.

**Statistical evaluation.** Results were expressed as means ± SE. An analysis of variance with Tukey-Kramer correction was used to examine differences between groups. P values < 0.05 were considered significant.

The research ethics boards at the Institute for Biodiagnostics of the National Research Council and at the University of Manitoba approved the above study. All subjects provided written informed consent before entering the study.

**RESULTS**

Four patients (three compensated and one decompensated) receiving ribavirin therapy for their hepatitis C were excluded from the study post hoc because of hepatic formation of the activated ribavirin metabolite ribavirin triphosphate (11). Once phosphorylated in hepatocytes, these nucleotide triphosphate analogs precluded determinations of hepatic ATP levels.

A summary of liver enzyme and function tests and Child-Pugh’s scores in the remaining 13 patients with compensated and decompensated cirrhosis are presented in Table 1. All patients with compensated cirrhosis were Child-Pugh’s A classification, whereas in those with decompensated cirrhosis, two were Child-Pugh’s A, one was Child-Pugh’s B, and three were Child-Pugh’s C. As predicted, patients with compensated cirrhosis...
rhosis tended to have earlier, more active liver disease (higher liver enzymes), whereas those with decompensated cirrhosis had more advanced hepatic dysfunction and a higher Child-Pugh’s score ($P = 0.01$). However, only serum albumin concentrations were significantly lower in those with decompensated cirrhosis ($P < 0.02$).

Figure 1 provides a typical axial image from a healthy volunteer. Indicated on the image is a voxel generated by the 2D-CSI pulse sequence from which localized spectral information from the liver is acquired. Expanded below is the localized hepatic $^{31}$P MR spectrum. A typical $^{31}$P MR spectrum from liver contains resonances belonging to PME, Pi, PDE, and the three phosphate groups ($\gamma$, $\alpha$, and $\beta$) from nucleotide triphosphates, the majority of which arise from ATP (14). PCr may also be detected in the in vivo spectrum of liver. This is indicative of signals arising from adjacent muscle, and its contribution is deducted from the final data output.

Representative $^{31}$P MR spectra obtained from a healthy control (Fig. 2A) and patients with compensated (Fig. 2B) and decompensated cirrhosis (Fig. 2C) are also shown. Hepatic $^{31}$P MR spectra from patients with compensated cirrhosis were equivalent to those seen in healthy controls.

As indicated in Table 2, significant metabolic derangements were evident in patients with decompensated cirrhosis compared with those with compensated cirrhosis and healthy controls, including lower levels of hepatic ATP ($P < 0.02$ and $P < 0.009$, respectively) and a higher PME/PDE ratio ($P < 0.003$ vs. healthy controls). However, hepatic ATP levels and PME/PDE ratios did not correlate with changes in Child-Pugh’s scores, serum albumin, bilirubin, or INR determinations.

DISCUSSION

To date, few studies have been performed that examine, at a cellular level, hepatocyte differences in patients with compensated vs. decompensated cirrhosis. In the present study $^{31}$P MRS was used to noninvasively assess the hepatic metabolic profile of such patients. Our findings suggest that hepatocytes within the decompensated cirrhotic liver have depleted energy stores and altered phospholipid metabolism compared with...
those within the livers of patients with compensated cirrhosis and healthy controls.

The mechanism whereby hepatic ATP levels become depleted in the decompensated state remains to be determined. Two possible explanations are 1) the loss of a critical amount of viable hepatocyte mass and/or 2) altered hepatic bioenergetics. With regards to the former, it has been well documented that the formation of cirrhosis involves repeated cycles of hepatic injury and repair that ultimately result in the deposition of fibrous tissue throughout the liver. When hepatocyte necrosis persists in the setting of inadequate liver regeneration (as occurs in decompensated cirrhosis) and extensive fibrosis extends across liver lobules, a significant loss of viable hepatocytes within the liver will occur (15, 19, 26). As the total amount of these cells per unit volume of liver decreases, the MR-detectable signal from that volume will also decrease. Previous experiments in our laboratory have demonstrated that reduced levels of hepatic ATP detected in animal models of acute and chronic liver disease are proportional to the diminished volume of viable liver tissue (6a, 6b).

Bioenergetic alterations may also influence hepatic ATP levels, because energy expenditure has been shown to increase with increasing severity of cirrhosis (42). This explanation is not incompatible with the diminished hepatocyte mass hypothesis, because disturbances in hepatic bioenergetics can arise from a diminished hepatocyte population within the cirrhotic liver. As the total volume of viable liver tissue decreases, residual hepatocytes must expend more energy to maintain hepatic function and engage in compensatory liver regeneration. Eventually, the remnant hepatocyte population is incapable of meeting these increasing demands, resulting in both energy depletion and hepatic insufficiency. Supporting this explanation are the results from studies wherein hepatic ATP levels correlated with the extent of hepatic dysfunction (7, 22, 23).

Altered hepatic bioenergetics have long been implicated as contributing to the disturbances associated with cirrhosis (4, 17, 36, 37). However, the present study is the first to report that hepatic ATP levels in vivo are significantly lower in patients with decompensated cirrhosis. Although previous quantitative $^{31}$P MRS studies (27, 38) have described decreased concentrations of hepatic ATP among alcoholic cirrhotics, patients in these studies were not stratified according to disease severity. In addition, concerns exist regarding the cause of ATP depletion in these individuals, because diminished levels were also detected in alcoholics with hepatitis who had not yet developed cirrhosis (27). To avoid such confounding variables, we selected a cohort of hepatitis C patients with cirrhosis and no alcohol intake for a minimum of 1 mo and off all medications that might adversely affect hepatocyte mitochondria and respiratory chain function.

The ratio of PME to PDE resonance in the $^{31}$P MR spectrum has traditionally been viewed as an index of cell membrane turnover (40). PME and PDE are both multicomponent resonances that contain contributions from metabolites involved in membrane synthesis [phosphocholine (PC); phosphoethanolamine (PE)] and degradation [glycerophosphocholine (GPC); glycerophosphoethanolamine (GPE)], respectively (30, 33, 34). Rapidly proliferating cells are likely to have high levels of PME relative to PDE. Indeed, high levels of PME/PDE have been reported in hepatic tumors (2) and in regenerating livers following hepatic resection (22). Several studies have documented elevations in hepatic PME/PDE with increasing severity of chronic hepatitis and cirrhosis (16, 20, 25, 43). In these studies, the increase has commonly been attributed to elevations in PME with concomitant reductions in PDE levels. Although in vitro MRS has confirmed that the concentrations of PC and PE increase in cirrhosis (43, 45), it is uncertain whether these elevations are sufficient to cause an increase in the total concentration of the PME resonance. In fact, increases in the absolute concentration of hepatic PME have not been reported in the cirrhotic liver (27, 38). In the present study, levels of hepatic PME were similar in the two study populations and controls. However, consistent with other reports, hepatic PME/PDE ratios were significantly elevated in those with decompensated cirrhosis. Hence, the increase in the ratio likely stemmed from lower levels of PDE, rather than increased PME. Although the drop in hepatic PDE only approached significance ($P = 0.05$) in patients with decompensated cirrhosis, it was sufficient to cause a significant shift in the PME/PDE ratio ($P < 0.003$).

The mechanism(s) whereby hepatic PDE levels falls in patients with decompensated cirrhosis is unclear. The two principle components of the PDE resonance include the degradative metabolites of phospholipid metabolism (GPC and GPE) and contributions from endoplasmic reticulum (ER) (32, 34). High resolution in vitro analysis has demonstrated that GPC and GPE concentrations are low in the cirrhotic liver (43, 45); however, these metabolites are only minor contributors to the PDE resonance at low magnetic field strengths (32). Thus a signal arising from the ER phospholipid membrane would be the major contributor to PDE resonance at clinical field strengths. As such, changes detected in the PDE resonance are likely to reflect disturbances in the ER. Indeed, electron microscopy studies have documented a striking decrease in the quantity of ER in hepatocytes from patients with decompensated cirrhosis compared with those with compensated disease (43).

Given the above, caution must be exercised when interpreting changes in the PME/PDE ratio. In settings where PME levels are increased, an elevation in the PME/PDE ratio is likely to be indicative of enhanced cell proliferation. In the present study, hepatic PME/PDE levels were increased in patients with decompensated cirrhosis; however, this increase may not have reflected enhanced cell proliferation (regeneration) but rather disturbances in the hepatic ER pool. The latter explanation would be more in keeping with the pathophysiol-

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**Table 2. Concentrations of hepatic phosphorus metabolites in healthy controls and hepatitis C patients with compensated and decompensated cirrhosis**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control Subjects (n = 8)</th>
<th>Compensated Cirrhosis (n = 7)</th>
<th>Decompensated Cirrhosis (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME</td>
<td>3.69 ± 0.56</td>
<td>4.74 ± 0.39</td>
<td>4.06 ± 0.49</td>
</tr>
<tr>
<td>P1</td>
<td>1.18 ± 0.07</td>
<td>1.15 ± 0.20</td>
<td>0.69 ± 0.21</td>
</tr>
<tr>
<td>PDE</td>
<td>7.64 ± 0.70</td>
<td>7.55 ± 1.08</td>
<td>4.53 ± 0.98</td>
</tr>
<tr>
<td>β-ATP</td>
<td>3.20 ± 0.15</td>
<td>3.11 ± 0.29</td>
<td>2.28 ± 0.20*</td>
</tr>
<tr>
<td>PME/PDE</td>
<td>0.47 ± 0.04</td>
<td>0.72 ± 0.12</td>
<td>1.07 ± 0.20*</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/l. PME, phosphomonoesters; PDE, phosphodiesters. *P < 0.05 vs. controls.
ogy of decompensated cirrhosis, because smaller hepatic ER pools may help explain the diminished drug-clearing capacity seen in decompensated cirrhosis. That the increased PME/PDE reflects enhanced regenerative activity is also unlikely given the high death rate from liver failure in these patients (9). It is important to note that the present study is not without caveats of its own. Absolute quantitation of tissue metabolites by MRS is a challenging task because many experimental variables must be controlled. The relaxation time constant of each metabolite, T1, is an important variable because it governs the metabolite peak area in the MR spectrum. Unfortunately, T1 values are difficult to determine in population studies, because 1) T1 may change in disease states and 2) long scan times are required for definitive measurements of T1. In the present study, T1 values from normal individuals were used for all subjects. This introduces a source of error to the calculated values of some metabolites, since certain metabolite T1 values change with cirrhosis (16, 25). Menon et al. (25) documented that T1 values of hepatic PME and Pi increase in cirrhosis, whereas PDE T1 values decrease. As a result, saturation effects would cause calculated concentrations of PME and Pi to be underestimated and PDE to be overestimated. Such errors may explain the discrepancy between the in vivo and in vitro reports discussed earlier. Interestingly, β-ATP T1 values remain unchanged between normal individuals and cirrhosis patients (16, 25); hence one can safely assert that the most striking change detected in the present study, the reduction in hepatic ATP levels among patients with decompensated cirrhosis, is both real and accurate.

In summary, the results of this study indicate that hepatic ATP and PDE levels are reduced in patients with decompensated but not compensated cirrhosis. The results also indicate that 31P MRS examination of the liver is a valuable tool for documenting the metabolic status of hepatocytes in patients with advanced liver disease.

ACKNOWLEDGMENTS

We thank Dr. V. Strevens and K. Hawkins for their assistance with patient accrual.

GRANTS

This work was supported by grants from the Health Sciences Centre Foundation, Canadian Liver Foundation, and the National Research Council of Canada, Institute for Diagnostics Investigative Initiative.

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