Magnetic Resonance Studies in Patients with Chronic Liver Disease and Following Hepatic Transplantation

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Abstract

Chronic liver disease is a major health problem and liver transplantation is the only form of treatment which prolongs life in end-stage liver disease. However, the current methods of assessing the functional severity of liver injury are not entirely satisfactory, usually depending on a collection of laboratory and clinical factors, which do not always directly reflect underlying hepatic function. Magnetic resonance spectroscopy (MRS) may provide direct biochemical information on hepatic metabolic processes. In this thesis, a combination of in vivo and in vitro phosphorus-31 MRS techniques are used to study liver function in patients with cirrhosis and also hepatic allograft rejection following liver transplantation. The results show that in vivo phosphorus-31 MR spectra vary with the functional severity of chronic liver disease. In vitro phosphorus-31 MRS demonstrates that the underlying biochemical abnormalities are due to changes in phospholipid metabolism and may be used as an indication of hepatic functional reserve. Patients with chronic ductopenic allograft rejection have spectral changes, probably reflecting altered phospholipid secretion into bile in this cholestatic condition. MR spectroscopy may provide an indication of bile duct damage, where early diagnosis is often difficult. A major complication of chronic liver disease is chronic hepatic encephalopathy, the neuropsychiatric abnormality which affects up to 80% of patients. The pathogenesis of the condition is unknown and it is difficult to monitor objectively. MRS may be utilised to study the biochemistry and metabolism of the brain. The cerebral function of encephalopathic patients with biopsy-proven cirrhosis of the liver is studied using standard clinical and electrophysiological techniques, which are compared with phosphorus-31 and hydrogen-1 (proton) MRS and MR imaging modalities. Results demonstrate spectral changes, which correlate closely with the underlying neuropsychiatric impairment, offering the possibility of studying the pathogenesis and providing an objective means of monitoring treatment modalities and patient responses to them.
DEDICATION

This study is dedicated to Dr Marsha Y. Morgan who fostered in me an enquiring mind.
ACKNOWLEDGEMENTS

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SUPERVISION

My supervisor for these studies was Professor Neil McIntyre from the University Department of Medicine, Royal Free Hospital and School of Medicine, Pond Street, London NW3 2QG. A cohort of liver transplant patients and those with chronic liver disease from the Royal Free Hospital were studied. Additional patients with chronic hepatic encephalopathy were recruited from the Hammersmith Hospital. All patients were entered into the studies and consented by me. I learnt how to operate the MR systems at the Hammersmith Hospital, how to process, analyse and interpret the data acquired. Liver samples for in vitro studies were obtained and processed by me. All studies outlined in this thesis form the basis either of already published work or else in press.
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<th>Description</th>
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<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Centigrade</td>
</tr>
<tr>
<td>CHE</td>
<td>chronic hepatic encephalopathy</td>
</tr>
<tr>
<td>Cho</td>
<td>choline</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>CSI</td>
<td>chemical shift imaging</td>
</tr>
<tr>
<td>¹³C MRS</td>
<td>carbon-13 magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>2,3-DPG</td>
<td>2,3-diphosphoglycerate</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetra-acetic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transformation</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>Glx</td>
<td>glutamine/glutamate</td>
</tr>
<tr>
<td>GPC</td>
<td>glycerophosphorylcholine</td>
</tr>
<tr>
<td>GPE</td>
<td>glycerophosphorylethanolamine</td>
</tr>
<tr>
<td>¹H MRS</td>
<td>proton magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>M</td>
<td>Molar concentration</td>
</tr>
<tr>
<td>mM</td>
<td>milliMolar concentration</td>
</tr>
<tr>
<td>MDP</td>
<td>methylene diphosphonate</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>mT</td>
<td>milliTesla</td>
</tr>
<tr>
<td>μT</td>
<td>microTesla</td>
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</tbody>
</table>
ABBREVIATIONS

MT magnetisation transfer
NAA n-acetylaspartate
NDP nucleotide diphosphate
NTP nucleoside triphosphate
NMR nuclear magnetic resonance
NRPB National Radiological Protection Board
OLT orthotopic liver transplantation
PC phosphocholine
PCA perchloric acid
PCr phosphocreatine
PDE phosphodiester
PE phosphoethanolamine
PET positron emission tomography
Pi inorganic phosphate
PME phosphomonoester
31P MRS phosphorus-31 magnetic resonance spectroscopy
PSE portal-systemic encephalopathy
rf radiofrequency
s seconds
SAR specified absorption ratio
SHR signal-height ratio
SI signal intensity
SNR signal-to-noise ratio
STEAM stimulated acquisition mode
T Tesla
T₁WSE T₁-weighted spin echo
TE echo time
TIPSS transjugular intrahepatic portasystemic stent shunt
TR repetition time
W Watt
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Chapter 1

THE PRINCIPLES OF MAGNETIC RESONANCE
1. THE PRINCIPLES OF MAGNETIC RESONANCE

1.1 HISTORICAL PERSPECTIVES

The nuclear magnetic resonance (NMR) phenomenon was first demonstrated experimentally in 1946 (Bloch et al., 1946; Purcell et al., 1946). This technique has been used widely by physicists and chemists ever since. The biomedical applications of NMR are twofold: magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). In the early 1970s, phosphorus-31 magnetic resonance spectra were obtained from isolated rat skeletal muscle (Hoult et al., 1974), while in the 1980s, the advent of wide-bore superconducting magnets made in vivo MR studies possible in man (Ross et al., 1981; Radda et al., 1989). Since its first human applications in the early 1980s, MRI has become an accepted and increasingly available clinical imaging method, while MRS may be used to obtain non-invasive biochemical information both on the composition of body fluids in vitro and on the function of animal and human organs in vivo.

1.2 PHYSICAL BASIS OF MAGNETIC RESONANCE

All isotopes with magnetic nuclei are capable of demonstrating the phenomenon of NMR. In reality this means that nearly all elements possess at least one isotope amenable to study. Of principal interest to the work in this thesis are the nuclei of hydrogen-1 (\(^1\)H) and phosphorus-31 (\(^31\)P). Other isotopes which may be employed in MR studies include carbon-13 (\(^13\)C), nitrogen-15 (\(^15\)N) and fluorine-19 (\(^19\)F), all of which can be induced to produce radiofrequency (rf) signals in the presence of a strong magnetic field (Gadian, 1982).

It should be noted that the natural abundance in the human body of the isotopes, hydrogen-1 and phosphorus-31, is actually or nearly 100\% (Table 1.1), but in contrast, by far the most
frequently occurring carbon isotope, carbon-12 (\(^{12}\text{C}\)), is not NMR sensitive. The isotope of interest, carbon-13, contributes just over 1% of the body’s total carbon nuclei (Table 1.1).

**Table 1.1: The natural abundance in the body of various NMR sensitive nuclei and their relative signal strengths**

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Natural abundance (%)</th>
<th>Relative sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protons ((^{1}\text{H}))</td>
<td>99.98</td>
<td>100.00*</td>
</tr>
<tr>
<td>Phosphorus ((^{31}\text{P}))</td>
<td>100.00</td>
<td>6.60</td>
</tr>
<tr>
<td>Carbon ((^{13}\text{C}))</td>
<td>1.11</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*since the \(^{1}\text{H}\) signal is the most intense, this has been arbitrarily assigned at 100.

**1.2.1 Static Magnetic Fields**

In a static magnetic field, (\(B_0\)), materials containing these nuclei become weakly magnetised along the direction of the field. \(B_0\) field strength is measured in Gauss (G) or Tesla (T) with 10,000G the equivalent of 1T. The strength of magnets used in most clinical MRI studies is 0.1T to 1.5T, which can be compared with the average strength of the Earth’s magnetic field which is approximately 0.5G or 0.00005T. However for MRS, higher magnetic field strengths of the order of 1.5T or greater are required for whole body human studies. Machines operating at 4.0T (Bomsdorf et al., 1988) are now available for both imaging and
spectroscopy in some centres such as Nottingham. With increasing magnetic field strength better spectral resolution is obtained and in vitro MRS of body fluids is often performed at up to 11.7T, far beyond the range of the wide-bore clinical systems employed in whole-body human studies.

1.2.2 Time Dependent Fields

An NMR signal is generated by applying a second magnetic field, \( B_1 \), which is orthogonal to \( B_0 \) and is also time dependent or oscillatory in nature. The peak field strength generated in \( B_1 \) is of the order of 50mT. Its effect is to rotate the direction of magnetisation from the longitudinal axis of the magnet (along \( B_0 \)) into the transverse direction. \( B_1 \) is usually applied in short pulses which rotate the magnetisation from its preferred alignment along \( B_0 \): rotations of 90° pulses orient the nuclei in the transverse plane; 180° pulses invert the magnetisation. On termination of the \( B_1 \) pulse, the magnetisation relaxes back to the original longitudinal direction. The decay of this excitation is the source of the NMR signal, which is modulated by the molecular environment of the MR sensitive nuclei under examination (Gadian 1982) (Figure 1.1).

1.2.3 Fourier Transformation

The spectral or frequency content of the signal, known as the free induction decay (FID), can be resolved by a mathematical process known as Fourier transformation (FT) into either an image, providing anatomical information for magnetic resonance imaging (MRI) or a frequency spectrum, providing biochemical information which is the basis of magnetic resonance spectroscopy (MRS) (Becker and Fisk, 1987) (Figure 1.2). The electronic and molecular interactions unravelled by FT are the root of the great diversity seen in MR studies.
1.2.4 Local Magnetic Fields

The magnetic environment of the MR sensitive nuclei is dominated by the externally applied fields, $B_0$ and $B_1$, but also has contributions by electrons in molecular binding and by neighbouring nuclei. These local magnetic fields determine the spectral appearances of the MR signal, being responsible for the phenomenon of chemical shift and also the linewidths or broadness of the resonances observed.

1.2.5 Chemical Shift

Whatever the chosen nucleus under MR examination, the nuclei from individual metabolites and molecular groups resonate at a given frequency, depending on the molecular structure or chemical environment of each compound or tissue, a phenomenon known as chemical shift (Figure 1.3). The frequency is dependent on $B_0$, so in order to be able to compare and contrast data obtained on different magnets, MRS results are presented in terms of chemical shift, expressed using the dimensionless unit, parts per million (ppm).

1.2.6 Relaxation Characteristics

The relaxation of the MR sensitive nuclei, following excitation by the $B_1$ pulse may be described by two processes, known as longitudinal and transverse relaxation. These are characterised by the time constants $T_1$ and $T_2$ respectively.

Longitudinal relaxation, expresses the behaviour of magnetisation components along $B_0$ as the nuclei return to equilibrium following the termination of the applied $B_1$ pulse. Before the next rf pulse sequence is applied, these nuclei should be fully relaxed in order to obtain quantitative information on the MRS visible metabolites present. If this is not allowed to happen, the signal is said to be partially saturated and quantification may therefore be
difficult, because individual metabolites have different relaxation times ($T_1$).

**Transverse** relaxation expresses the loss in the transverse components of magnetisation following the termination of a $B_1$ pulse. Stronger transverse relaxation causes the MRS metabolite resonances to appear broader and thus the shorter the $T_2$, the broader these MRS metabolite resonances appear (Gadian, 1982).

1.3 MAGNETIC RESONANCE IMAGING

Conventional MRI employs the signal from only one MR sensitive nucleus, hydrogen-1, present in body water and fat to demonstrate anatomy and structural pathology. The $^1$H signals are acquired in the presence of magnetic field gradients, which allow full spatial localisation of the signal. These gradients, $G_x$, $G_y$ and $G_z$, vary in three spatial dimensions: the x axis (left to right or the transverse direction), y axis (anteroposteriorly or the sagittal direction) and the z axis (longitudinally or the craniocaudal direction). The MR signals from the different parts of the body area being imaged are encoded with spatial information, each $^1$H nucleus resonating at a slightly different frequency owing to the three dimensional variation in magnetic field strength. This allows signal from different points to be assigned to different pixels in the computer reconstructed image matrix.

The appearance of the MR image is dependent on the characteristics of the $B_1$ pulses used to detect the signal. Series of $B_1$ or "pulse sequences" can be altered to highlight different anatomical structures preferentially and to provide a potent method of soft tissue image contrast. On $T_1$-weighted imaging, which emphasises differences in longitudinal relaxation, tissues with a short $T_1$ such as fat appear bright, while tissues with a longer $T_1$, such as muscle appear darker. Using such $B_1$ pulse sequences, fluids which are mobile and have a longer $T_1$, appear darker than solids. In $T_2$-weighted images, transverse relaxation
differences are emphasised. Tissues with a short $T_2$, such as muscle appear darker and those such as bile or urine with a high signal intensity and a longer $T_2$ are brighter.

### 1.3.1 Magnetisation Transfer

Magnetisation transfer (MT) is another mechanism for manipulating tissue contrast (Wolff and Balaban, 1989). Tissue $^1$H nuclei (protons) exist in two different compartments. The first consists of free, mobile protons such as those in body water. As described above, these nuclei have a relatively long relaxation times ($T_1$ and $T_2$) and provide the majority of the signal which is detected by the conventional clinical whole body MR systems. The second grouping of protons are those which are components of, or are bound to proteins, cell membranes and other large macromolecules. These nuclei have a very short $T_2$ and if conventional MR imaging techniques are used, signal arising from these protons is not directly detectable and is said to be MR invisible (Wolff and Balaban, 1989; Hajnal et al., 1992). However, magnetisation can transfer between the bound and free protons. Substantial changes in tissue contrast may be obtained by using pulse sequences which affect the tissue bound protons, while leaving the free protons in the body water unaffected. This involves the use of an off-resonance rf pulse which nullifies the magnetisation in the bound proton pool, so that there is very little or no transfer of magnetisation from the bound nuclei into the free proton pool in the body water. By this process, the apparent $T_1$ of the free protons is substantially reduced. Therefore these changes in tissue contrast may be used to define anatomy and highlight pathological changes differently to standard MR sequences (Hajnal et al., 1992).
1.4 MAGNETIC RESONANCE SPECTROSCOPY

Unlike MRI, MRS makes use of one of a number of other NMR sensitive nuclei. Currently, $^{31}\text{P}$ and $^1\text{H}$ MRS are used in a wide variety of applications in the brain and $^3\text{P}$ MRS for functional information on the liver, forming the basis for the work in this thesis. Signals from compounds of concentrations of the order of 1-10mM or greater are detected with these MRS techniques.

Data from $^{13}\text{C}$ are often much more difficult to obtain, mainly because this nucleus has a natural abundance of 1% of the body’s carbon, whereas the most abundant carbon isotope, $^{12}\text{C}$ is NMR insensitive. Therefore, the $^{13}\text{C}$ signal intensity is weak, being of the order of 5,000 times less than that from body water (Table 1.1) and requiring magnets of increased field strength (1.5 Tesla and above) to obtain the MR data. Naturally occurring $^{13}\text{C}$ is present in high enough concentrations in adipose tissue for in vivo MRS (Moonen et al., 1988; Bryant et al., 1993), but most studies augment the NMR signal by using $^{13}\text{C}$ labelled tracers such as glucose to follow metabolic pathways in vivo (Rothman et al., 1992).

As with MRI, where tissue contrast may be highlighted by altering the characteristics of the applied $B_1$ pulse sequences, editing of MR spectral appearances can also be achieved by $B_1$ pulse sequence manipulation.

1.4.1 In vitro MR Spectroscopy

Body fluids and tissue extracts may be examined using in vitro MR spectroscopic techniques at much higher magnetic field strengths than the wide-bore whole body clinical systems used in vivo. The in vitro MR spectra are better resolved than those obtained from whole body tissue at 1.5T, not only because of the higher fields, but also because samples are physically spun to remove residual imperfections in the homogeneity of the $B_1$ field. These
well resolved spectra may therefore allow characterisation of the biochemical changes observed in vivo.

1.4.2 Metabolite Quantification

The intensity of each metabolite signal is related to its concentration (Figure 1.3), but in practice absolute quantification is difficult to achieve for a number of reasons. These include both $T_1$ and $T_2$ effects. A more rigorous account is given by Bottomley (Bottomley, 1991).

Many in vivo MRS studies therefore report results in terms of metabolite ratios. Analysis of the MR spectrum allows non-invasive insight into metabolite concentrations, intracellular pH, the metabolic state of pathological tissue and also dynamic changes in the metabolism of living tissue in both health and disease (Cady, 1990). However, only compounds present in millimolar concentrations are detectable utilising in vivo MRS with the clinical MR systems currently available. (Cady, 1990).

1.5 MAGNETIC RESONANCE METHODS

The hardware for an MR system includes a magnet, rf coils for transmitting and receiving the rf signal, magnetic field gradients for anatomical signal localisation, a spectrometer and a computer system for data collection and processing. The arrangement is essentially the same for both MR spectroscopy and imaging and many systems operating at 1.5T and above are equipped for both.

1.5.1 The Magnet

Most of the clinical systems currently used for both MRI and in vivo MRS employ cryogenic superconducting magnets. Human in vivo hepatic and cerebral MRS studies have
mostly been performed using horizontal bore magnets with a magnetic field strengths in the range of 1.5 to 2.0T, but higher spectral resolution particularly for $^{13}$C and $^{31}$P MRS studies has been demonstrated utilising 4T systems (Bomsdorf et al., 1988; Barfuss et al., 1990). Vertical bore magnets with a field strength of 4.7 to 9.0 T are used for in vivo animal studies on a routine basis, while in vitro MRS studies make use of field strengths of up to 11.7T for fine spectral metabolite resolution of the components of tissue extracts or body fluids (Bell et al., 1993).

1.5.2 RF Coils

The size and shape of the transmitter and receiver coil arrangements are usually governed by the area of the body under examination. Thus specifically designed coils are available for MRI and MRS examinations of the head and different areas of the body.

1.5.2.1 Transmitter Coils

The applied $B_1$ pulse is usually provided by an enveloping transmitter coil, which surrounds the patient in order to provide a uniform magnetic field and rf pulse angle throughout the area of the body being studied. This is required for studies where the "field of view" (FOV) is important, such as in MRI or in multivoxel MRS techniques, where information from different anatomical regions are collected simultaneously. All the work in this thesis employs such techniques.

Some studies have however, employed a surface transmitter coil, which simply overlies the area of the body under examination such as the liver. The magnetic field inhomogeneities that result from this type of coil can be substantially improved by tailoring the rf pulse appropriately (Bendall et al., 1983).
1.5.2.2 Receiver Coils

The receiver coil consists of a conducting loop of wire, which is usually placed over the surface of the body to pick up the rf signal from the patient. Electronic hardware amplifies and processes the signal. Surface coils are very sensitive over a small region close to the coil. In other words, their FOV is limited, because they acquire signal from only a restricted volume of tissue. However, in many studies receiver coils have been combined with enveloping transmitter coils to produce a transmit-receive system. Such arrangements are user friendly and often make patient positioning easier.

1.5.3 The Computer System

The computer system is responsible for controlling the electronic hardware which produces the pulse sequences used in both MR imaging and MR spectroscopy. It is also important for the acquisition, processing and storage of the MR data acquired (Gadian, 1982).

1.6 THE PATIENT EXAMINATION

MRI and MR spectroscopy involve the use of high magnetic field strength superconducting magnets, exposing patients to potential injury from items made of magnetisable material (Kanal et al., 1990). It is therefore imperative to strictly exclude patients or staff members with any form of loose metal from the vicinity of the magnet. Exclusion criteria must also extend to individuals with cardiac pacemakers and ferrometallic implants such as surgical clips. Exclusion criteria for research examinations also extend to pregnant women.

There is no ionising radiation involved and the magnetic fields strengths used in human studies are thought to be intrinsically safe. However rf fields do provide heating within the body, through the induction of eddy currents. Rf field exposure should be such as to avoid
a rise in body temperature of more than 0.5°C. This has lead to guidelines limiting the specified absorption ratio (SAR) experienced by human subjects. In the UK, the NRPB guidelines are a body average SAR of 0.4W/kg and a peak of SAR of 4W/kg in any 1g of tissue (Guidelines of the National Radiological Protection Board, 1991). All the studies outlined in this thesis are within these NRPB guidelines.

1.6.1 Patient Positioning and Magnet Shimming

A typical clinical examination takes about one hour. The subject lies on a bed, while the transmitter and receiver rf coils are positioned and is then moved into the bore of the magnet. The region of interest should lie centrally in the magnet, where the B₀ field is most uniform. The magnetic field in the region of interest is then optimised for each patient, a process known as shimming, which may take a few minutes.

Patient comfort is vital for the acquisition of good spectra. Discomfort is invariably associated with movement, which causes distortions in the magnetic field, detuning of the rf coils, and alterations in tissue content of the selected region of interest, resulting in poor quality images and making MR spectral localisation artefacted.

1.7 DATA ACQUISITION

At the beginning of the MR spectroscopy examination, a series of MR images are usually obtained to visualise the appropriate anatomy within the magnet coordinates. Signal excitation is achieved by the application of a short rf pulse, the nature of which depends on the frequency range of the nucleus of interest. The utilisation of magnetic field gradients allows spatial localisation of spectra. Such techniques include single voxel methods such as ISIS (Ordidge et al., 1986) or multivoxel techniques, such as chemical shift imaging (CSI).
Signals from successive radiofrequency pulses are added together or "averaged" in order to improve sensitivity. If absolute quantitation of metabolites is to be achieved, the pulse repetition time (TR) needs to be 5 times longer than the T₁. However, this is time consuming and extends the examination time considerably. Therefore, with patient comfort in mind, a compromise TR value is normally used.

The time between signal excitation with the rf pulse and the start of data acquisition is known as the echo time or TE. Ideally, the TE should be as short as possible so that no data is lost, but in some sequences the TE is deliberately longer. To facilitate spatial localisation with gradient pulses, the TE may have to be increased. In the case of proton spectroscopy, longer TE also help to attenuate the strong signal from unwanted water and particularly lipid resonances.

### 1.7.1 Spectral Analysis

The MR signal in the form of the FID is usually filtered to reduce noise levels and contributions from broad overlapping resonances, before Fourier transformation to produce a spectrum. The data obtained can be analysed to obtain relative or absolute concentrations of metabolites, the individual resonances in each MR spectrum being examined by peak area analysis using computer software. However, absolute metabolite quantitation is difficult to achieve (Young et al., 1989; Bottomley, 1991) and therefore peak areas are usually referenced to an internal or external reference standard. An external reference consists of a standard solution in a vial situated outside the body, but within the FOV of the transmitter and receiver rf coil. An internal reference is a stable metabolite occurring naturally in the tissue such as ATP. For in vitro MRS studies, compounds can be introduced into the tissue extract or body fluid sample at an accurately known concentration.
1.8 References


Figure 1.1:

DIAGRAMMATIC REPRESENTATION OF THE PRINCIPLES OF MAGNETIC RESONANCE:

a) MR sensitive nuclei possess the property of spin and when not subject to a magnetic field, they precess randomly about their own axes.

b) When placed in a stationary magnetic field ($B_0$) these nuclei all realign along its axis.

c) When a radiofrequency pulse is then applied, in the form of an oscillating magnetic field ($B_1$), the MR nuclei are perturbed out of the original axis in (b) to a new one.

d) At the termination of the radiofrequency pulse, the nuclei return to their original position (b), giving off a radiofrequency signal, which may then be detected by a receiver coil.
(a) Stationary magnetic field

(b) Oscillating magnetic field

(c) Receiver coil

(d) NMR signal
The MR signal or free induction decay (FID) may be converted by the mathematical process of Fourier transformation to form anatomical information (MR imaging) or localised biochemical information (MR spectroscopy).
Figure 1.3:

DIAGRAM ILLUSTRATING THE PRINCIPLES OF CHEMICAL SHIFT:

a) All MR sensitive nuclei (protons) in a bottle of water are subject to the same chemical environment. They therefore resonate at a uniform frequency and the MR spectrum consists of only one peak.

b) However, MR sensitive nuclei in any more complicated compound resonate at slightly different frequencies, which depend on the chemical structure of the surrounding nuclei. For example, in a bottle of alcohol, there are three resonances arising from the three parts of the chemical compound. Since there are three times as many protons in the CH$_3$- group as in the -OH group, the signal of the former is three times the intensity of the latter.
a

Intensity ↑

Frequency F

Spectrum consists of one line

b

Intensity ↑

Frequency CH₃-CH₂-OH

Spectrum consists of three lines
AN INTRODUCTION TO HEPATIC MAGNETIC RESONANCE SPECTROSCOPY
2. AN INTRODUCTION TO HEPATIC MAGNETIC RESONANCE SPECTROSCOPY

2.1 Measures of Hepatocellular Injury

Irrespective of the original causal agent, the liver responds to injury in broadly the same way, with alteration both to its structural and functional organisation. The degree of injury is conventionally assessed using tests which reflect its structure (tissue biopsy), cellular permeability (serum transaminase concentrations) and synthetic activity (prothrombin time, plasma albumin and serum bilirubin levels). The histological analysis of liver biopsy material is useful diagnostically, but is a poor discriminant in the provision of prognostic indicators of patient survival. The measurement of serum transaminases does not reflect the functional capacity of the liver, while measures of synthetic activity are of limited value early in the disease process, because of the large functional reserve of the liver. Furthermore, considerable variation may occur in plasma albumin levels, depending upon the nutritional state of the patient (Jalan and Hayes, 1995).

2.1.1 Dynamic Liver Function tests

Dynamic tests of liver function such as indocyanine green clearance (Skak and Keiding, 1987), mono-ethyl-glycine-xylidide (MEGX) formation (Oellerich et al., 1991), galactose elimination capacity (Aebli and Reichen, 1991), antipyrine clearance (Homeida et al., 1979), the aminopyrine breath test (Villeneuve et al., 1986) and caffeine clearance (Renner et al., 1984) have not found universal usefulness in predicting outcome in chronic liver disease.
2.1.2 The Child-Pugh Scoring Systems

The most widely used index of the functional severity of chronic liver disease is the Child classification or its modifications (Child and Turcotte, 1964; Pugh et al., 1973). This scoring system is based upon a collection of laboratory parameters and subjective clinical findings, which are dependent on a number of extra-hepatic influences including patient treatment. These make it an imprecise model in predicting outcome in individual patients.

2.2 Hepatic MR Spectroscopy

*In vivo* hepatic magnetic resonance spectroscopy (MRS) is a non-invasive technique, which can be used to study metabolic processes in man. Phosphorus-31 has been the most widely applied nucleus in MRS studies of the liver, providing information on intracellular concentrations of metabolites critical to cell function. Signals from metabolites which reflect rates of cell membrane synthesis and breakdown and also cellular energy states can be obtained using $^{31}$P MRS (Bottomley, 1989; Aisen and Chenevert, 1989).

2.2.1 The *In Vivo* Phosphorus-31 MR Spectrum

The $^{31}$P MR spectrum acquired from the liver of a healthy volunteer *in vivo* contains six major resonances, representing the phosphomonoesters (PME), inorganic phosphates (Pi), phosphodiesters (PDE) and the $\alpha$, $\beta$ and $\gamma$ moieties of the nucleotide triphosphates (NTP) (Cox et al., 1992). Analysis of these resonances provide information on cellular bioenergetics and intracellular pH (Taylor et al., 1983) the metabolism of membrane phospholipids (Ruiz-Cabello and Cohen, 1992) and carbohydrates (Dagnelie et al., 1992) and also magnesium concentrations (Gupta and Moore, 1980).
The PME, PDE and NTP resonances are multicomponent and the constituents are not fully separable at the magnetic field strengths employed in human in vivo MRS studies, despite the use of proton-decoupling techniques. Changes in these resonances may be related to alterations in the levels of a number of metabolites (Cox et al., 1991).

2.2.2 Hepatic Energy Status and Measurement of Intracellular pH.

Alterations in the hepatic energy state is reflected by changes in the absolute quantity of high energy phosphates and also the MRS ratio of NTP to either the total phosphate metabolites measured or to inorganic phosphate. It should be noted that the NTP resonances are not composed solely of adenosine triphosphate (ATP), but are also contributed to in part by uridine triphosphate and guanosine triphosphate (Iles et al., 1985). The α NTP resonance also contains contributions from α adenosine diphosphate and nicotinamide adenine dinucleotide, while the γ NTP resonance contains a contribution from β ADP.

An assessment of intracellular pH can be made indirectly as the chemical shift of Pi is dependent upon the pH (Cohen et al., 1982). However, not all of the intracellular inorganic phosphates are MR visible, owing to binding of Pi in the mitochondria (Iles et al., 1985).

2.3 Hepatic Phosphorus-31 MR Spectroscopy in Chronic Liver Disease

Although a number of studies have been performed to evaluate the role of $^{31}$P MRS in the assessment of the severity and prognosis of chronic liver disease, the results are not directly comparable because most studies were small, the populations of patients examined were heterogeneous and the MRS methods employed varied widely.

Meyerhoff and co-workers studied eight patients with alcoholic cirrhosis with no significant change reported in the PME/ATP or PDE/ATP ratios. However, the mean PME/ATP ratio
was increased by 25% and the mean PDE/ATP was reduced by 12% (Meyerhoff et al., 1989), while Angus and colleagues studied five abstinent patients with alcoholic cirrhosis, with no significant change in the PME/ATP and PDE/ATP ratios reported. Like the previous study, the mean PDE/ATP ratio was reduced by 12% (Angus et al., 1990).

Oberhaensli and colleagues found a significant reduction in the Pi/ATP ratio in three patients with primary biliary cirrhosis (PBC), compared with healthy volunteers (Oberhaensli et al., 1990), while Cox and colleagues reported a significant inverse relationship between the PME/PDE ratio and albumin concentration in 25 patients with diffuse liver disease of varying aetiology (Cox et al., 1992).

None of these studies categorised patients according to the severity of liver injury. However, Munakata and colleagues (Munakata et al., 1993) studied 14 abstinent patients with alcoholic cirrhosis of varying functional grade, classified on the basis of both the Child's grading system (Child and Turcotte, 1964; Pugh et al., 1973), and the results of the aminopyrine breath test (Villeneuve et al., 1986). These authors showed a significant relationship between the severity of liver disease and an elevation in the PME resonance, expressed as a percentage of the total $^3$P signal. A similar reduction in the PDE resonance was observed in this group of patients, but this did not reach statistical significance.

The most definitive hepatic $^3$P MRS study was performed by Menon and his colleagues, comprising 85 patients with cirrhosis of differing disease aetiology and functional grade, again classified according to the Child's grading system (Menon et al., 1995). These investigators demonstrated elevations in the PME/ATP and PME/PDE ratios, which correlated directly with increasing functional severity of liver disease, while reductions in the PDE/ATP ratio correlated inversely with these clinical indices. Differences were also observed in spectral appearances in relationship to the aetiology of liver injury, but these
were only obvious in those with compensated liver disease, rather than with decompensated disease. Therefore, in patients with hepatic injury in its early stages, post viral cirrhosis was associated with a significant increase in the Pi/ATP ratio, alcoholic cirrhosis with reduced a PDE/ATP ratio and primary sclerosing cholangitis (PSC) with a reduced Pi/ATP ratio, compared with patients with other liver disease of similar functional severity.

2.3.1 Implications

These observations by Menon and colleagues have two important implications:

First, $^3$P MRS may be used successfully as a diagnostic tool for assessment of the aetiology of liver disease in the early stages and the results also suggest that the technique may be used to predict patient outcome, because the changes in the $^3$P MR spectrum correlate with the severity of liver disease.

The second inference that may be made is that although liver diseases of varying aetiology start differently, ultimately they produce the same end result in terms of functional injury in the decompensated liver.

At the higher magnetic field strengths employed by in vitro MRS techniques, the composite resonances observed in vivo may be fully defined. Therefore, further studies using in vitro MR spectroscopy are required to define the biochemical changes underlying the in vivo hepatic MRS changes seen in patients with chronic liver disease.
2.4 Liver Transplantation

OLT is the treatment of choice for end stage liver failure, but up to 15% of recipients subsequently develop chronic graft rejection. This is characterised by biliary stasis and progressive loss of bile ducts (Wiesner et al., 1993). Early diagnosis is crucial for optimising immunosuppression, which may arrest the pathological process that may otherwise ultimately result in graft failure and retransplantation.

Liver biopsy is the diagnostic "gold standard", but because the liver may be affected unevenly, serial biopsies are often required in order to observe the pathological changes with any degree of confidence.

Phosphorus-31 MR spectroscopy may be of diagnostic potential in this clinical situation and could possibly provide insight into the pathogenesis of the condition.
HEPATIC MAGNETIC RESONANCE SPECTROSCOPY
IN LIVER DISEASE:

2.5 AIMS

THE AIMS OF THIS STUDY WERE THREEFOLD:

1. To characterise the nature of the \textit{in vivo} hepatic $^{31}\text{P}$ MRS abnormalities in chronic liver disease using \textit{in vitro} MRS techniques. 

\textit{(The results of this study are detailed in chapter 3).}

2. To correlate \textit{in vivo} hepatic $^{31}\text{P}$ MRS changes in patients with chronic liver disease with \textit{in vitro} $^{31}\text{P}$ MRS of liver extracts taken at the time of orthotopic liver transplantation.

\textit{(The results of this study are detailed in chapter 4).}

3. To investigate the changes in $^{31}\text{P}$ MR spectra in patients postoperatively with good allograft function and with chronic ductopenic rejection.

\textit{(The results of this study are detailed in chapter 5).}
2.6 References


IN VITRO PHOSPHORUS-31 MR SPECTROSCOPY OF LIVER EXTRACTS
3. IN VITRO PHOSPHORUS-31 MR SPECTROSCOPY OF LIVER EXTRACTS

3.1 Abstract

Human livers with histologically proven cirrhosis were assessed using in vitro $^{31}$P MR spectroscopy. Spectra were compared with those from histologically normal livers and showed significant elevations in phosphoethanolamine (PE) and phosphocholine (PC) and significant reductions in glycerophosphorylethanolamine (GPE) and glycerophosphorylcholine (GPC). There were no significant differences in spectra from livers with compensated and decompensated cirrhosis. These results help to characterise the alterations in membrane metabolism in cirrhosis of the liver.

3.2 Introduction

The human liver responds to injury in broadly the same way, irrespective of the original causal agent (Sherlock and Dooley, 1993). Persistent alcohol abuse, viruses such as hepatitis B and hepatitis C, genetic disorders including haemochromatosis, Wilson's disease and $\alpha_1$-antitrypsin deficiency, cholestatic conditions such as primary biliary cirrhosis and primary sclerosing cholangitis, certain drugs and autoimmune diseases all may provoke a series of events that ultimately lead to cirrhosis or irreversible liver damage (Erlinger and Benhamou, 1991).

Cirrhosis of the liver is a diffuse process, characterised by the formation of fibrous tissue and regrowth of hepatocytes in an abnormal nodular pattern (Sherlock and Dooley, 1993).
Current assessment methods of the functional state of liver injury in cirrhosis are not entirely satisfactory, usually depending on a severity index obtained from a collection of laboratory parameters and clinical findings (Pugh et al., 1973; Albers et al., 1989; Dickson et al., 1989).

Magnetic resonance spectroscopy is a non-invasive technique, which can be used to provide localised biochemical information on hepatic metabolic processes in vivo. A typical $^{31}$P MR spectrum of the human liver in vivo contains resonances which may be assigned to phosphomonoesters (PME), phosphodiesters (PDE), inorganic phosphate (Pi) and nucleotide triphosphates (NTP) (Cox et al., 1988).

The PME and PDE resonances in hepatic spectra are multicomponent and the constituents cannot as yet be completely resolved at the magnetic field strengths employed in human in vivo MRS studies. The PME resonance includes contributions from cell membrane precursors (Ruiz-Cabello and Cohen, 1992) and glycolytic intermediates (Bell et al., 1993). The PDE resonance is also composite, containing information from cell membrane breakdown products (Ruiz-Cabello and Cohen, 1992) and from endoplasmic reticulum (Murphy et al., 1989).

Previous human in vivo MRS studies have reported on the elevation in PME/ATP and the reduction in PDE/ATP with increasing functional severity of cirrhosis (Munakata et al., 1993; Menon et al., 1995). However, the underlying metabolic abnormalities responsible for these observations have not been fully investigated.

In vitro MR spectroscopy techniques on human tissue extracts have been successfully used to study the metabolite changes responsible for the in vivo PME and PDE signals in hepatic tumours and normal liver (Bell et al., 1993; Cox et al., 1992a). However, no systematic approach has been applied to the characterisation of the cirrhotic liver.
3.3 Aims

Therefore, the aim of this study was to characterise the metabolic changes observed by \textit{in vitro} $^{31}\text{P}$ MRS in cirrhosis of the liver. The results are discussed in the context of previous \textit{in vivo} hepatic $^{31}\text{P}$ MRS findings.

3.4 Materials and Methods

Standard percutaneous liver biopsies do not yield enough tissue for \textit{in vitro} MRS studies, and therefore samples of cirrhotic liver were taken during surgery for orthotopic hepatic transplantation. Liver tissue was obtained from 25 patients with histologically proven cirrhosis. Ten patients (40\%) had primary biliary cirrhosis, seven (28\%) post-viral cirrhosis, six (24\%) primary sclerosing cholangitis, one (4\%) Wilson's disease and one (4\%) alcoholic cirrhosis.

The severity of liver dysfunction was assessed using the Pugh's score, obtained from clinical and biochemical data, acquired on the day of liver transplantation. This is the standard scoring system, which is used clinically, grading liver injury from 5 (best function) to 15 (worst function), taken from information comprising serum bilirubin, plasma albumin levels, prothrombin time and the presence/severity of ascites and hepatic encephalopathy (Pugh \textit{et al.}, 1973).

The 25 liver samples were categorised into two groups: functionally compensated cirrhosis with a Pugh's score of less than or equal to 7 ($n=10$) and functionally decompensated cirrhosis with a Pugh's score greater than or equal to 8 ($n=15$) (Table 3.1).

Permission for this study was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London and the Royal Free Hospital and School of Medicine, London. All patients provided written, informed consent.
3.4.1 Sample Collection

Tissue samples were obtained from each of the 25 recipient livers. In every case, six to eight representative sugar lump sized pieces of liver were freeze-clamped in liquid nitrogen with minimum possible ischaemic time (2-7 minutes). This was performed ex vivo within 3 minutes of hepatectomy in 22 of the 25 cases. All samples were stored separately in a liquid nitrogen dewar until further processed.

3.4.2 Reference Data

Reference data were obtained from wedge biopsy samples of liver, taken from six patients undergoing laparotomy for surgical treatment of pancreatitis. In each case, contiguous samples of liver tissue were found to be histologically normal on examination (Bell et al., 1993).

3.4.3 Tissue Extract Preparation

The wet weight of each sample was between 560 mg and 2310 mg. Twelve per cent perchloric acid (PCA) was added to the still-frozen samples, in a ratio of 5 ml/g of liver tissue. Each sample was ground down under liquid nitrogen with a mortar and pestle and then allowed to thaw, before centrifugation at 3000 rpm for 10 minutes. The supernatant was separated, neutralized with 3 M potassium hydroxide, freeze-dried and reconstituted in deuterium oxide. The pH was readjusted to 7.5, after the addition of 100 \( \mu \text{mol} \) of ethylenediaminetetra-acetic acid (EDTA) to chelate any paramagnetic metal ions present. Absolute quantification of metabolites was achieved by adding known amounts of methylene diphosphonate (MDP) and/or phosphocreatine (PCr) to the perchloric acid extracts. These acted as internal reference standards for chemical shift assignments of the resonances observed.
3.4.4 *In Vitro* MRS Methods

All MR spectroscopy measurements were performed at room temperature. Proton-decoupled $^{31}$P MR spectra were obtained using a high resolution MR spectroscopy system (operating at 11.7T), from the perchloric acid extracts of liver tissue, with 16K data points and a 45° pulse angle applied at intervals of 1s. Corrections for $T_1$ relaxation were made using samples run with a repetition time of 20s. Metabolites were assigned using the standard methods previously described (Bell *et al.*, 1993); the chemical shift of each metabolite was found and subsequently confirmed by the use of "spiking" with known compounds.

3.4.5 Data Processing

The free induction decay (FID) was zero filled to 32K and Fourier transformed after line-broadening of 5 Hz. Peak areas for PE, PC, GPE, GPC, MDP and/or PCR were obtained, using the NMR1® spectral processing program (New Methods Research, Inc., E. Syracuse, U.S.A.) on a SUN SPARCstation 10 (Sun Microsystems, Inc., Mountain View, CA, U.S.A.). The data were fitted to Lorentzian functions.

3.4.6 Statistical Analysis

Since the data were not normally distributed, non-parametric statistical analysis was applied. Values for metabolite concentrations in the patient and reference populations were compared using the Mann Whitney U test. A p value of <0.05 was considered significant. All metabolite concentrations are quoted as mean values $\pm 1$ standard deviation.
3.5 Results

A typical $^3$P MR spectrum from a PCA extract of normal liver contains resonances arising from PME, PDE, NTP, NDP and Pi (Figure 3.1). The PME region of the spectrum consists of over 10 resonances, including signal from PE, PC, adenosine monophosphate (AMP), coenzyme A, 2,3-diphosphoglycerate (2,3-DPG) and sugar phosphates such as glucose 6-phosphate, glycerol 3-phosphate, glycerol 1-phosphate, 3-phosphoglycerate and ribose 5-phosphate (Cox et al., 1992a; Bell et al., 1993). The PDE region contains two major resonances, GPE and GPC (Cox et al., 1992a; Bell et al., 1993).

Most of these resonances vary markedly with ischaemia and it was therefore only sensible to quantify the more stable compounds, namely PE and PC from the PME region and GPE and GPC from the PDE region of the spectrum (Dawson, 1955; Perry et al., 1971).

The signal intensity of the PE and PC resonances was increased and the GPE and GPC resonances reduced in spectra from liver with histologically proven cirrhosis (Figure 3.2), when compared to spectra from histologically normal liver. The metabolite concentrations (pmol/g wet weight of liver tissue) are summarised in Table 3.2.

All cirrhotic livers showed significantly higher PE (1.04±0.75 vs 0.16±0.03; p<0.0005) and PC concentrations (0.41±0.37 vs 0.16±0.04; p<0.05) and significantly lower GPE (0.29±0.37 vs 2.35±0.46; p<0.005) and GPC concentrations (0.14±0.26 vs 2.46±0.37; p<0.0001) than normal tissue (Table 3.2).

There was no significant difference between PE, PC, GPE and GPC concentrations from livers with functionally compensated cirrhosis and those from livers from functionally decompensated cirrhosis (Table 3.2).

There were regional variations in metabolite concentrations when liver samples from different areas of the same liver were analysed. Table 3.3 illustrates these variations in...
metabolite levels in a patient with compensated cirrhosis.

There was no correlation between individual biochemical indices (serum bilirubin, plasma albumin and prothrombin time) or clinical parameters of liver dysfunction (presence of ascites and hepatic encephalopathy), measured on the day of the transplant operation, and PE, PC, GPE and GPC concentrations from the liver extracts.

3.6 Discussion

This study used in vitro $^{31}$P MR spectroscopy to describe the changes in aqueous soluble membrane components in livers with histologically proven cirrhosis, compared to normal human liver tissue.

Several human in vivo $^{31}$P MR spectroscopy studies of the liver have shown abnormalities in PME, PME/ATP, PME/PDE and PDE/ATP in patients with cirrhosis (Meyerhoff et al., 1989; Cox et al., 1992b; Rajanayagam et al., 1992; Munakata et al., 1993; Menon et al., 1995). Two of these studies have correlated the functional severity of liver injury in cirrhosis with an elevation in PME/ATP and a reduction in PDE/ATP (Munakata et al., 1993; Menon et al., 1995).

This study attempted to investigate the underlying metabolic changes responsible for these in vivo spectral appearances in man. Unfortunately, a limitation of human tissue characterisation by in vitro methods is the unavoidable period of ischaemia during biopsy collection. Only quantification of PE, PC, GPE and GPC was attempted, as the other metabolites that comprise the PME and PDE peaks are known to alter radically from the in vivo situation during periods of ischaemia (Cox et al., 1992a, Bell et al., 1993). Hachisuka and colleagues noted that in rat liver subjected to prolonged periods of ischaemia beyond 30 minutes, PC and PE were relatively stable, while GPE and GPC decreased (Hachisuka et al.,
1992). However, post-mortem studies of human brain and animal liver have indicated that the levels of PE, PC, GPE and GPC are not significantly affected by periods of ischaemia of up to one hour (Dawson, 1955; Perry et al., 1981). In the current study much shorter periods of ischaemia were encountered. Twenty-two of the 25 tissue samples from cirrhotic liver were collected within 3 minutes of hepatectomy, while in the three tissue samples the ischaemic period was up to 7 minutes.

Comparison of the $^{31}$P MR spectra of PCA extracts from cirrhotic liver and histologically normal tissue showed increased concentrations of PE and PC and decreased concentrations of GPE and GPC from the diseased tissue. Regional variations in metabolite concentrations were observed from samples obtained from different areas of each individual liver.

These results suggest that increased concentrations of PE and PC may be responsible for elevation in PME/ATP observed in vivo (Rajanayagam et al., 1992; Munakata et al., 1993; Menon et al.; 1995). Similarly, the reduction of PDE/ATP seen in vivo (Munakata et al., 1993; Menon et al.; 1995) may be explained, at least in part by the reductions in GPE and GPC which were noted in this present study. Endoplasmic reticulum is also an important component of the PDE resonance in vivo (Bates et al., 1989; Murphy et al., 1989), but its relative contribution in the human cirrhotic liver is unclear and requires further investigation.

The predominant contribution of PC and PE are as intermediates on the pathway of phospholipid biosynthesis while GPE and GPC are phospholipid breakdown products (Ruiz-Cabello and Cohen, 1992). Increased PE (Morikawa et al., 1992; Murphy et al., 1992) and PC (van Noorden et al., 1988) have been observed in the regenerating rat liver and in other conditions of rapid cellular proliferation, such as in hepatic tumours (Cox et al., 1992a, Bell et al., 1993; Dixon and Tian, 1993). Lymphomatous infiltration of the liver is also associated with elevated PE levels (Dixon and Tian, 1993).
The hallmark of cirrhosis is abnormal regrowth of liver tissue in a nodular pattern. This occurs in the presence of increased fibroblastic activity (Sherlock and Dooley; 1993). The increase in PE and PC in the present study may therefore be due to increased cell turnover as the cirrhotic liver attempts to regenerate. Either hepatocyte regeneration or the laying down of fibrous tissue, during the cirrhotic process, may be responsible for this phenomenon.

GPE and GPC levels are reduced in rapidly proliferating cells (Cox et al., 1992a; Morikawa et al., 1992; Bell et al., 1993; Farghali et al., 1994) in conditions of increased cell turnover such as the failing cirrhotic liver, it may be reasonable to expect reduced levels of these cell membrane degradation products.

Unlike the published in vivo studies, where there was an elevation in PME/ATP and PDE/ATP which correlated with the functional severity of liver injury (Munakata et al., 1993; Menon et al.; 1995), there was no statistical difference between metabolite levels from functionally compensated and functionally decompensated cirrhotic liver in this in vitro study. This may partially reflect the arbitrary nature of the clinical grading system (Pugh et al., 1973), which is subject to a number of extrahepatic influences.

Furthermore, the regional variation in metabolites concentrations that were observed within each individual liver, highlight the fact that cirrhosis is not a uniform process. Therefore, the lack of distinction between liver samples from patients with compensated and decompensated cirrhosis may also be a reflection of the varying composition of these tissue samples.

Further studies correlating in vivo 31P MR spectral abnormalities with in vitro 31P MR spectroscopy appearances and electron microscopy of liver tissue to assess the MR contribution of endoplasmic reticulum are required. However, the results of this study suggest that the changes in PE, PC, GPE and GPC are responsible, to a large extent, for the PME/ATP and PDE/ATP abnormalities seen in patients with cirrhosis of the liver.
3.7 References


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**Table 3.1.** Laboratory data on the patients from whom the liver samples were collected, mean (range values)

* limits of reference range.

| Tissue type | Serum bilirubin (µmol/L) (5-17)* | Plasma albumin (g/L) (35-50)* | Prothrombin time (s) (12-14)* | Pugh's score *
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</thead>
<tbody>
<tr>
<td>Compensated (n=10)</td>
<td>177 (35-460)</td>
<td>40 (31-48)</td>
<td>14 (13-16)</td>
<td>7 (6-7)</td>
</tr>
<tr>
<td>Decompensated (n=15)</td>
<td>143 (34-388)</td>
<td>31 (22-40)</td>
<td>17 (13-25)</td>
<td>10 (8-12)</td>
</tr>
</tbody>
</table>

*Pugh's score (Pugh et al., 1973) = functional severity of cirrhosis.
Score ≤ 7 = compensated cirrhosis. Score ≥ 8 = decompensated cirrhosis.
All information was obtained pre-operatively, on the day of liver transplantation.
Table 3.2. Concentrations of metabolites obtained from in vitro $^{31}$P MR spectra from histologically normal and cirrhotic liver tissue. (mean values ±1SD)

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Metabolite concentrations (µmol/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
</tr>
<tr>
<td>Normal liver (n=6)</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>All cirrhosis (n=25)</td>
<td>1.04 ± 0.75$^a$</td>
</tr>
<tr>
<td>Compensated cirrhosis (n=10)</td>
<td>1.28 ± 0.70$^a$</td>
</tr>
<tr>
<td>Decompensated cirrhosis (n=15)</td>
<td>0.88 ± 0.76$^d$</td>
</tr>
</tbody>
</table>

significant difference from the reference population:

$^a$ p <0.0005, $^b$ p <0.05, $^c$ p <0.0001, $^d$ p <0.001, $^e$ p <0.01
Table 3.3 In vitro $^{31}$P MRS: Variations in metabolite concentration obtained from different regions of the same liver

<table>
<thead>
<tr>
<th>Metabolite concentration</th>
<th>Region 1</th>
<th>Region 2</th>
<th>Region 3</th>
<th>Region 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>0.09-0.24*</td>
<td>1.34</td>
<td>1.76</td>
<td>3.72</td>
</tr>
<tr>
<td>PC</td>
<td>0.11-0.23*</td>
<td>0.43</td>
<td>0.77</td>
<td>0.91</td>
</tr>
<tr>
<td>GPE</td>
<td>1.79-2.71*</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>GPC</td>
<td>2.09-2.83*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All values expressed as μmol/g wet weight of liver tissue

*reference range
Figure 3.1:
Typical proton-decoupled $^3$P MR spectrum of perchloric acid extract prepared from histologically normal liver tissue.
(a) Full spectrum;
(b) PME and PDE regions.
Abbreviations: PME = phosphomonooesters; PDE = phosphodiesters; NAD = NADH + NAD;
NTP = nucleotide triphosphates; NDP = nucleotide diphosphate.
PE = phosphoethanolamine, PC = phosphocholine, GPE = glycerophosphorylethanolamine,
GPC = glycerophosphorylcholine, PCr (phosphocreatine) was added as an internal reference standard.
Figure 3.2
Typical proton decoupled $^{31}$P NMR spectrum of perchloric acid extract from liver tissue with histologically proven cirrhosis:
(a) Full spectrum,
(b) PME and PDE regions.
Abbreviations: PME = phosphomonoesters; PDE = phosphodiesters; NAD = NADH + NAD; NTP = nucleotide triphosphates; NDP = nucleotide diphosphate.
PE = phosphoethanolamine, PC = phosphocholine, GPE = glycerophosphorylethanolamine, GPC = glycerophosphorylcholine, PCr (phosphocreatine) and MDP (methylene diphosphonate) were added as internal reference standards.
IN VIVO - IN VITRO CORRELATIONS IN HEPATIC $^{31}$P MRS IN PATIENTS WITH CHRONIC LIVER DISEASE
4. **IN VIVO – IN VITRO CORRELATIONS IN HEPATIC $^{31}$P MRS IN PATIENTS WITH CHRONIC LIVER DISEASE**

4.1 Summary

The aim of this study was to observe the changes in the *in vivo* $^{31}$P MR spectra prior to liver transplantation in a cohort of patients with chronic liver disease and to characterise the underlying abnormalities using *in vitro* $^{31}$P MRS of recipient liver tissue obtained at transplantation.

The study included 31 patients with biopsy-proven cirrhosis of varying aetiology; 14 had compensated cirrhosis (Pugh's score < 7) and 17 patients had decompensated cirrhosis (Pugh's score > 8). One dimensional chemical shift imaging techniques were used to obtain *in vivo* $^{31}$P MR spectra localised to the liver. Peak area ratios of phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr) relative to $6^{\text{ATP}}$ (ATP) and PME/PDE were measured in spectra acquired at 1.5 Tesla. *In vitro* $^{31}$P MR spectra were obtained at 11.7 Tesla from perchloric acid extracts of recipients' liver tissue, freeze-clamped at the time of orthotopic liver transplantation. Phosphoethanolamine (PE), phosphocholine (PC), glycerophosphorylethanolamine (GPE) and glycerophosphorylcholine (GPC) were measured.

An elevation in PME/ATP and reduction in PDE/ATP was observed with increasing functional severity of liver injury *in vivo*. The *in vitro* spectra showed a rise in PE and PC and reduction in GPE and GPC which mirrored the changes seen *in vivo*, but no clear distinction was noted between compensated and decompensated cirrhosis *in vitro*.

The *in vivo* $^{31}$P MR spectral abnormalities seen in end stage liver disease are due to changes in phospholipid metabolism. The variations in metabolite ratios with increasing severity of liver damage may prove useful as a non-invasive test of functional reserve.
4.2 Introduction

The response of the liver to injury is relatively predictable in histological terms. A wide variety of causal agents including alcohol, hepatotropic viruses, drugs, genetic and autoimmune disorders may provoke the same series of events that ultimately lead to cirrhosis. However, the clinical spectrum of liver injury is broad, extending from asymptomatic hepatomegaly to profound functional decompensation with jaundice, ascites and portal hypertension (Sherlock and Dooley, 1993). Adequate characterisation of the functional state of liver injury in cirrhosis is essential for management purposes. Current assessment methods of the functional grade of cirrhosis are not entirely satisfactory, usually depending on a severity index obtained from a collection of laboratory parameters and clinical findings (Child et al., 1964; Pugh et al., 1972; Dickson et al., 1989). These scoring systems may be subject to extrahepatic influences that reduce their value as indicators of liver function. Other grading systems used for prognostic information are based on Cox models. They include histological analysis of liver biopsy material and may require serial liver biopsies with all the attendant risks of this procedure (Christensen et al., 1985). Dynamic liver function tests such as galactose elimination capacity (Aebli and Reichen, 1991) and the aminopyrine breath test (Villeneuve et al., 1986) are dependent on hepatic blood flow and may be relatively insensitive indicators of hepatic functional reserve (Jalan and Hayes, 1995).

In vivo MR spectroscopy is a non-invasive technique, which can be used to provide localised biochemical information on hepatic metabolic processes. A typical $^{31}$P MR spectrum of the human liver in vivo contains resonances which may be assigned to phosphomonoesters (PME), phosphodiesters (PDE), inorganic phosphate (Pi) and nucleotide triphosphates (NTP) (Oberhaensli et al., 1986; Cox et al., 1988; Meyerhoff et al., 1989b; Angus et al., 1990; Oberhaensli et al., 1990).
Several human *in vivo* $^{31}\text{P}$ MR studies of the liver have shown abnormalities in PME, PME/ATP, PME/PDE and PDE/ATP in patients with cirrhosis (Meyerhoff *et al.*, 1989a; Cox *et al.*, 1992a, Rajayanayagam *et al.*, 1992; Munakata *et al.*, 1993; Menon *et al.*, 1995). Two of these studies have correlated the functional severity of liver injury in cirrhosis with an elevation in PME/ATP and a reduction in PDE/ATP (Munakata *et al.*, 1993; Menon *et al.*, 1995). The underlying metabolic abnormalities responsible for these observations were investigated in the previous chapter.

Both the PME and PDE resonances are multicomponent and their constituents cannot be completely resolved using clinical MR systems, despite the use of proton-decoupling techniques. However, at higher magnetic field strengths, *in vitro* MRS techniques provide the required spectral definition to resolve these metabolites. The PME resonance includes contributions from phosphocholine (PC), and phosphoethanolamine (PE), intermediates on the pathway of phospholipid membrane synthesis (Ruiz-Cabello and Cohen, 1992) as well as contributions from adenosine monophosphate (AMP) and glycolytic intermediates (Bell *et al.*, 1993). The PDE resonance is also composite, containing information from glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), intermediates on the pathway of phospholipid breakdown (Ruiz-Cabello and Cohen, 1992) and a contribution from endoplasmic reticulum (Murphy *et al.*, 1989).

### 4.3 Aims

The aims of the study were to correlate the changes in *in vivo* $^{31}\text{P}$ MR spectra in patients with liver disease of varying functional severity and differing aetiology, prior to orthotopic liver transplantation with abnormalities observed by *in vitro* MRS examination of freeze-clamped liver samples obtained from these patients at the time of recipient hepatectomy.
4.4 Patients and Methods

The study population comprised 31 individuals (16 men, 15 women) with biopsy-proven cirrhosis of mean (range) age 46.3 (21 - 68) years, all of whom were assessed for orthotopic liver transplantation. Twenty-two patients (71%) had biliary cirrhosis, seven (23%) post-viral cirrhosis, one (3%) alcoholic cirrhosis and one (3%) cryptogenic cirrhosis. As in the previous chapter, liver function in the patients in this study was assessed using the Pugh's score (Pugh et al., 1973), obtained from data acquired on the day of MRS study (Table 4.1). Patients were divided into two groups: functionally compensated cirrhotics with a Pugh's score ≤7 and functionally decompensated cirrhotics with a Pugh's score ≥8. All patients were abstinent from alcohol for a minimum of three months prior to the MRS study. The mean type interval between in vivo MRS examination and transplantation was 33 days (range 6 - 161 days).

The reference population comprised 18 healthy volunteers (11 men, seven women) of mean (range) age 38.1 (25-58) years, all of whom consumed less than 20 g daily of alcohol and none was taking regular medication.

All studies were performed after an overnight fast. Individuals were excluded from the study if they were claustrophobic or had cardiac pacemakers, other ferromagnetic implants or were known to be pregnant. All patients and volunteers provided written, informed consent. Permission for this study was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC/4047) and the Royal Free Hospital and School of Medicine, London.
4.4.1 *In Vivo* MRS Methods

*In vivo* hepatic $^{31}$P MR spectra were obtained from each patient, prior to orthotopic liver transplantation, using a Picker prototype spectroscopy system (Picker International, Cleveland, Ohio, USA), based on a whole body magnet (Oxford Magnet Technology, Oxford, UK), operating at 1.5 Tesla. An enveloping transmitter coil and separate surface receiver coil were used, both of which were double-tuned for protons at 64 MHz and phosphorus at 26 MHz. The proton signal was used for shimming and to obtain a $T_1$-weighted image in the axial plane to confirm spectral localisation. A one dimensional chemical shift imaging technique (1-D CSI) was used to acquire localised spectra from eight parasagittal planes, each of nominal width 30mm (Bailes *et al.*, 1987). Data were collected with a repetition time (TR) of 5000ms and a pulse angle of 45°. The pulse was calibrated using an external pick-up loop, which monitored the radio-frequency (rf) field directly. The variations in loading upon the transmitter system between phantom calibration studies and *in vivo* human studies were corrected for by this procedure. The choice of a 45° radiofrequency (rf) is commensurate with improving spectral appearances in instances where TR is sufficiently low that resonances are partially saturated. The total examination time varied between 45 and 60 minutes.

4.4.2 *In Vivo* Data Processing

A single observer, blinded to the patient's clinical status (S.D.T.-R.), processed and analysed all the data. Spectra were processed with a cosine filter in the spatial domain and a 60 ms exponential (or 5 Hz Lorentzian) filter in the time domain. After Fourier transformation, all spectra were manually phased. The delay in applying the localising phase-encoding gradients in the 1-D CSI sequence resulted in the appearance of a baseline roll. This artefact was
removed using knowledge-based data processing (Saeed and Menon, 1993). Peak area ratios for PME, Pi, PDE, βATP (ATP) and PME/PDE in the baseline corrected spectra were measured using the NMR1® spectral integration program (New Methods Research, Inc., E. Syracuse, NY) on a SUN SPARCstation 10 (Sun Microsystems, Inc., Mountain View, CA, USA). The spectra were fitted to inverse polynomial functions and the resulting metabolite ratios were subsequently correlated with the Pugh's score for each individual patient, obtained from data acquired on the day of in vivo MRS study.

4.4.3 Statistical Analysis

The 95% confidence intervals for the individual metabolite ratios in the healthy volunteers were used to define the reference range and values outside this range were considered abnormal. Non-parametric statistical analysis were used throughout the study, because the data were not normally distributed. Values for the metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test. Comparisons between patient subgroups were made using the Mann Whitney U test with a Bonferroni correction, where necessary. A p value of <0.05 was considered to be significant in all cases.

4.5 In Vitro Methods

The methods used were the same as in the previous chapter. All liver samples for in vitro MRS were collected from patients on the Royal Free Hospital liver transplant programme. All liver extracts were examined on a 11.7T magnet at the N.I.M.R. at Mill Hill.
4.5.1 Sample Collection.

Samples of cirrhotic liver were taken at recipient hepatectomy, during surgery for orthotopic hepatic transplantation, from each of the 31 patients who had undergone \textit{in vivo} MRS. In every case, six to eight representative pieces of liver, each approximately of $1\text{cm}^3$ in size, were freeze-clamped in liquid nitrogen with minimum possible ischaemic time (2-9 minutes). This was performed \textit{ex vivo} within 3 minutes of hepatectomy in 27 cases. All samples were stored separately in a liquid nitrogen dewar until further processed.

Liver function on the day of liver transplantation was again assessed using the Pugh's score (Pugh \textit{et al.}, 1973), obtained from clinical and biochemical data, acquired immediately prior to the operation (Table 2.2). On this basis, the liver samples from the 31 patients were then categorised into two groups: functionally compensated cirrhosis with a Pugh's score $\leq 7$ ($n=14$) and functionally decompensated cirrhosis with a Pugh's score $>8$ ($n=17$).

The same reference data were used as in the previous study, described in chapter 3. These results were obtained from wedge biopsy samples of liver, taken from patients undergoing laparotomy for surgical treatment of pancreatitis. In each case, contiguous samples of liver tissue were found to be histologically normal on examination (Bell \textit{et al.}, 1993).

4.5.2 Sample Processing.

In each case, one to two grams of liver tissue were processed with 12\% perchloric acid (PCA), added to the still-frozen samples, in a ratio of 5 ml/g of liver. This procedure ensured complete extraction of water soluble metabolites. Each sample was ground down under liquid nitrogen with a mortar and pestle and then allowed to thaw, before centrifugation at 3000 rpm for 10 minutes. The supernatant was separated, neutralized with 3 M potassium hydroxide, freeze-dried and reconstituted in deuterium oxide. The pH was
readjusted to 7.5, after the addition of 100|µmol of ethylenediaminetetra-acetic acid (EDTA) to chelate any metallic ions present, such as Cu²⁺ or Fe²⁺, which might otherwise degrade the quality of the signal obtained. Absolute quantification of metabolites was achieved by adding known amounts of the reference substances, methylene diphosphonate (MDP) and/or phosphocreatine (PCr) to the perchloric acid extracts. These acted as internal reference standards for chemical shift assignments of the resonances observed.

4.5.3 In Vitro MRS Methods.

All MRS measurements were performed at room temperature. Proton-decoupled ³¹P MR spectra were obtained using a 11.7 Tesla Bruker MR spectroscopy system, from perchloric acid extracts of the freeze-clamped tissue samples, with 16K data points and a 45° pulse angle applied at intervals of 1s to 20s.

4.5.4 Data Processing

The free induction decay (FID) was zero filled to 32K and Fourier transformed after line-broadening of 5 Hz. Peak areas for PE, PC, GPE, GPC, MDP and/or PCr were obtained, using the NMR1® spectral integration program, which fitted the data to Lorentzian functions.

4.5.5 Statistical Analysis

Since the data were not normally distributed, non-parametric statistical analysis was applied. Values for metabolite concentrations in the patient and reference populations were compared using the Mann Whitney U test. A p value of <0.05 was considered significant. All metabolite concentrations are quoted as mean values ±1 standard deviation.
4.6 Results

4.6.1 In vivo MRS

The in vivo hepatic $^{31}$P MR spectrum contains at least six resonances (Bates et al., 1989) which can be assigned to phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), $\gamma$ ATP, $\alpha$ ATP and $\beta$ATP (ATP) (Figure 4.1). The $\alpha$ ATP peak contains contributions from $\alpha$ ADP and NADH and the $\gamma$ ATP contains contributions from $\beta$ADP. A small resonance arising from phosphocreatine (PCr) is present in certain spectra because the MR localisation techniques do not always completely exclude signals from anterior and posterior abdominal wall muscle.

The same resonances were seen in the in vivo hepatic $^{31}$P MR spectra obtained from the patient population (Figure 4.2), but there were significant differences in the metabolite ratios compared to healthy individuals. The mean PME/ATP ratio was significantly elevated in the total patient population ($p<0.05$) (Table 4.3) and the mean PDE/ATP ratio was significantly reduced ($p<0.05$) (Table 4.3). The mean derived PME/PDE ratio from the 31 cirrhotic patients was therefore elevated considerably ($p<0.0005$), when compared to the reference population (Table 4.3).

When patients were classified as having compensated or decompensated cirrhosis, there was an increase in the PME/ATP ratio with the severity liver dysfunction (Table 4.4). The mean PME/ATP ratio in the 17 patients with decompensated cirrhosis was significantly different from both healthy volunteers ($p<0.0005$) and patients with compensated cirrhosis ($p<0.005$) (Table 4.4). However, there were no significant differences in mean PME/ATP ratios between healthy volunteers and the 14 patients with compensated cirrhosis (Table 4.4).

The PDE/ATP ratio varied inversely with the functional severity of the liver disease (Table 4.4). The mean PDE/ATP ratio in the 17 patients with decompensated cirrhosis was
therefore significantly reduced when compared to healthy volunteers (p<0.05), but not to patients with compensated cirrhosis (Table 4.4). There was also no significant difference in mean PDE/ATP ratios between healthy volunteers and the 14 patients with compensated cirrhosis (Table 4.4).

Furthermore, the derived ratio of PME to PDE increased with worsening liver function (Table 4.4). In patients with decompensated cirrhosis, the mean PME/PDE ratio was significantly different from both healthy volunteers (p<0.0005) and patients with compensated cirrhosis (p<0.005) (Table 4.4). However there was no statistically significant difference between this metabolite ratio when the 18 healthy volunteers and the patients with compensated cirrhosis were compared (Table 4.4).

There were no significant differences in the mean Pi/ATP ratios between the reference population and the either the total patient population (Table 4.3) or the patient subgroups (Table 4.4).

4.6.2 In vitro MRS

A typical in vitro $^{31}$P MR spectrum from a PCA extract of normal liver contains resonances arising from PME, PDE, NTP, NDP and Pi (Figure 4.3). The PME region of the spectrum consists of over 10 resonances, including signal from PE, PC, AMP, coenzyme A, 2,3-DPG and sugar phosphates such as glucose 6-phosphate, glycerol 3-phosphate, 3-phosphoglycerate and ribose 5-phosphate (Bell et al., 1993). The PDE region contains two major resonances, GPE and GPC (Cohen, 1983, Bell et al., 1993).

Many of these metabolites, such as the intermediates of carbohydrate metabolism, vary markedly with ischaemia and it was therefore only sensible to quantify the more stable compounds, namely PE and PC from the PME region and GPE and GPC from the PDE
region of the spectrum (Dawson, 1955; Perry et al., 1971; Perry et al., 1981).

The signal intensity of the PE and PC resonances was increased and the GPE and GPC resonances reduced in spectra from all 31 patients with cirrhosis (Figure 4.4), when compared to spectra from the six histologically normal livers. The metabolite concentrations (μmol/g wet weight of liver tissue) are summarised in Table 4.5.

There were significantly higher PE (p<0.0005) and PC concentrations (p<0.005) and significantly lower GPE (p<0.005) and GPC concentrations (p<0.0005) in the PCA extracts from all 31 patients than from the six reference livers (Table 4.5).

There was no significant difference between PE, PC, GPE and GPC concentrations from the 14 livers with functionally compensated cirrhosis and those from the 17 livers with functionally decompensated cirrhosis (Table 4.5). Both groups had significantly different metabolite concentrations from the reference levels (Table 4.5).

4.7 Discussion

The in vivo hepatic 31P MR spectra from patients with cirrhosis showed significant differences in metabolite ratios with respect to the severity of liver injury, the PME/ATP and PME/PDE ratios rising and the PDE/ATP ratio falling with worsening hepatic function.

Previous human in vivo 31P MR studies of the liver have also shown abnormalities in PME, PME/ATP, PME/PDE and PDE/ATP in patients with cirrhosis (Meyerhoff et al., 1989a; Cox et al., 1992b, Rajayanayagam et al., 1992; Munakata et al., 1993; Menon et al., 1995). Two of these studies correlated the functional severity of liver injury in cirrhosis with an elevation in PME/ATP and a reduction in PDE/ATP (Munakata et al., 1993; Menon et al., 1995). Munakata and colleagues (Munakata et al., 1993) studied 14 patients with cirrhosis of differing aetiology and found that there was an elevation in PME according to functional
grade, based on the Pugh's modification of the Childs' classification (Pugh et al., 1973) and the aminopyrine breath test. Menon and colleagues studied a much larger series of 85 patients and 16 healthy volunteers (Menon et al., 1995). The elevation in PME/ATP they observed, in relation to the functional severity of liver injury, was thought to represent increased phospholipid synthesis in the rapidly regenerating liver, while the reduction in PDE/ATP was thought to represent a combination of altered phospholipid metabolism and reduced signal from hepatic endoplasmic reticulum.

This present study used in vitro MRS to correlate the underlying changes in membrane metabolism responsible for the in vivo spectral appearances in this cohort of patients with cirrhosis. Only quantification of PE and PC, intermediates on the pathway of phospholipid biosynthesis, and the phospholipid breakdown products, GPE and GPC was performed, because the relative amounts of other metabolites contributing to the PME and PDE resonances are known to change even during the short periods of ischaemia in this study (Bell et al., 1993). However, post-mortem studies of human brain and animal liver indicate that the levels of PE, PC, GPE and GPC are not significantly affected by periods of ischaemia of up to one hour (Dawson, 1955; Perry et al., 1971; Perry et al., 1981).

The in vitro $^{31}$P MR spectra from PCA extracts of liver tissue obtained at the time of transplant hepatectomy in this cohort of 31 patients revealed an elevation in PE and PC and a reduction in GPE and GPC, which mirrored the elevation in PME/ATP and the reduction in PDE/ATP seen in vivo in the preoperative period. The increase in PE and PC in the liver extracts from patients in this study may be due to increased cell turnover as the cirrhotic liver attempts to regenerate (Murphy et al., 1992), while the reduction in GPE and GPC levels is expected in common with previous studies on rapidly proliferating cells, because these metabolites are recirculated back into the synthetic pathways (Cox et al., 1992a; Morikawa et al., 1992; Bell et al., 1993; Farghali et al., 1994).
There were no clear distinctions between the measured metabolite levels in liver tissue from patients classified as having compensated cirrhosis and those classified as having decompensated cirrhosis. This may reflect the inadequacies of the clinical grading system (Pugh et al., 1973), which is subject to a number of extrahepatic influences. However, cirrhosis of the liver is not a uniform process and therefore the in vitro liver extracts may have been subject to a degree of sampling error, since the in vivo results are composite information from a large area of liver, but the in vitro data are from 1-2g representative samples of tissue, taken immediately after recipient hepatectomy. The results from the previous chapter, showing a regional variation in metabolite concentrations from the same liver (Table 3.3), imply strongly that this latter supposition is correct.

The contributions of other metabolites such as glycolytic intermediates to the PME signal are not assessable in human in vitro studies, owing to instability with minimal ischaemic insults (Hems and Brosnan; 1970). Similarly, endoplasmic reticulum (ER) is also an important component of the PDE resonance in vivo (Bates et al., 1989; Murphy et al., 1989).

In order to fully resolve the question of the reduction in the PDE/ATP ratio observed in vivo in these patients, electron microscopy studies are required to quantify the amount of ER in end-stage liver disease.

However the results of this study, correlating in vivo and in vitro hepatic MR spectroscopy in a cohort of patients undergoing liver transplantation, suggest that the changes in PE, PC, GPE and GPC are responsible, to a large extent, for the PME/ATP and PDE/ATP abnormalities seen in patients with cirrhosis of the liver. The variation of metabolite ratios with in vivo hepatic spectra with respect to liver injury, suggests that 31P MR spectroscopy may be of use in the functional grading of liver disease and because it is not blood flow dependent or subject to extrahepatic influences, unlike other grading systems or dynamic liver function tests, it may provide useful information for the timing of liver transplantation in individual patients.
4.8 References


Table 4.1 Liver function tests, prothrombin time and Pugh's score of study patients on the day of *in vivo* hepatic $^{31}$P MRS examination mean (range) values

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Serum aspartate transaminase (u/L) (5-40)*</th>
<th>Serum alkaline phosphatase (u/L) (35-130)*</th>
<th>Serum bilirubin (μmol/L) (5-17)*</th>
<th>Plasma albumin (g/L) (35-50)*</th>
<th>Prothrombin time (s) (12-14)*</th>
<th>Pugh's score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensated cirrhosis (n=14)</td>
<td>161 (82-236)</td>
<td>935 (110-3063)</td>
<td>177 (35-460)</td>
<td>40 (31-48)</td>
<td>14 (12-15)</td>
<td>6-7</td>
</tr>
<tr>
<td>Decompensated cirrhosis (n=17)</td>
<td>172 (40-626)</td>
<td>324 (90-1133)</td>
<td>143 (25-388)</td>
<td>31 (22-40)</td>
<td>16 (13-25)</td>
<td>8-14</td>
</tr>
</tbody>
</table>


bcompensated cirrhosis = Pugh's score ≤ 7
cdecompensated cirrhosis = Pugh's score ≥ 8
Table 4.2  Liver function tests, prothrombin time and Pugh's score of the study patients on the day of transplantation mean (range) values

<table>
<thead>
<tr>
<th>Patient group (n)</th>
<th>Serum aspartate transaminase (u/L) (5-40)*</th>
<th>Serum alkaline phosphatase (u/L) (35-130)*</th>
<th>Serum bilirubin (μmol/L) (5-17)*</th>
<th>Plasma albumin (g/L) (35-50)*</th>
<th>Prothrombin time (s) (12-14)*</th>
<th>Pugh's score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensated cirrhosis(^b) (n=14)</td>
<td>157 (92-263)</td>
<td>820 (160-2150)</td>
<td>138 (23-293)</td>
<td>39 (31-49)</td>
<td>14 (12-15)</td>
<td>6-7</td>
</tr>
<tr>
<td>Decompensated cirrhosis(^c) (n=17)</td>
<td>150 (42-461)</td>
<td>317 (93-1111)</td>
<td>155 (34-610)</td>
<td>33 (27-40)</td>
<td>17 (13-25)</td>
<td>8-12</td>
</tr>
</tbody>
</table>

\(^a\)degree of functional impairment (Pugh et al., 1973).
\(^b\)compensated cirrhosis = Pugh's score ≤ 7
\(^c\)decompensated cirrhosis = Pugh's score ≥ 8
Table 4.3  *In vivo* hepatic $^{31}$P MRS metabolite ratios in the patient and reference populations.

Mean (± 1 SD) values

<table>
<thead>
<tr>
<th>Study group (n)</th>
<th>PME/ATP</th>
<th>Pi/ATP</th>
<th>PDE/ATP</th>
<th>PME/PDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers (n=18)</td>
<td>0.85±0.25</td>
<td>0.86±0.31</td>
<td>3.86±0.71</td>
<td>0.22±0.06</td>
</tr>
<tr>
<td>All patients (n=31)</td>
<td>1.44±0.75*</td>
<td>1.00±0.54</td>
<td>3.24±1.06*</td>
<td>0.52±0.40**</td>
</tr>
</tbody>
</table>

Significant difference from reference population: *p <0.05; **p <0.0005
Table 4.4  *In vivo* hepatic ³¹P MRS metabolite ratios in the patient subgroups and the reference population.

<table>
<thead>
<tr>
<th>Study group</th>
<th>PME/ATP</th>
<th>Pi/ATP</th>
<th>PDE/ATP</th>
<th>PME/PDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers (n=18)</td>
<td>0.85±0.25</td>
<td>0.86±0.31</td>
<td>3.86±0.71</td>
<td>0.22±0.06</td>
</tr>
<tr>
<td>Compensated cirrhosis* (n=14)</td>
<td>0.95±0.29</td>
<td>0.79±0.32</td>
<td>3.74±1.03</td>
<td>0.28±0.13</td>
</tr>
<tr>
<td>Decompensated cirrhosis^ (n=17)</td>
<td>1.84±0.78*</td>
<td>1.18±0.63</td>
<td>2.84±0.93*</td>
<td>0.73±0.11*</td>
</tr>
</tbody>
</table>

*compensated cirrhosis = Pugh's score ≤7 (Pugh et al., 1973).
^decompensated cirrhosis = Pugh's score ≥8 (Pugh et al., 1973).

Significant difference from healthy volunteers: *p<0.05; †p<0.005

Significant difference from patients with compensated cirrhosis: ▼ p<0.005
Table 4.5 Metabolite concentrations obtained from *in vitro* MRS of liver tissue.

mean (± 1 SD) values

<table>
<thead>
<tr>
<th>Study group</th>
<th>PE</th>
<th>PC</th>
<th>GPE</th>
<th>GPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal liver</strong> (n=6)</td>
<td>0.16±0.03</td>
<td>0.16±0.04</td>
<td>2.35±0.46</td>
<td>2.46±0.37</td>
</tr>
<tr>
<td><strong>All cirrhosis</strong></td>
<td>1.37±0.86</td>
<td>0.45±0.35*</td>
<td>0.31±0.47❖</td>
<td>0.19±0.43❖</td>
</tr>
<tr>
<td>Compensated cirrhosis* (n=14)</td>
<td>1.41±0.76</td>
<td>0.35±0.16*</td>
<td>0.22±0.28❖</td>
<td>0.09±0.18❖</td>
</tr>
<tr>
<td>Decompensated cirrhosisb (n=17)</td>
<td>1.34±0.96</td>
<td>0.53±0.43†</td>
<td>0.38±0.58❖</td>
<td>0.26±0.55❖</td>
</tr>
</tbody>
</table>

All values expressed as µmol/g wet weight of liver tissue

*compensated cirrhosis = Pugh's score ≤ 7 (Pugh *et al.*, 1973).

*bdecompensated cirrhosis = Pugh's score ≥ 8 (Pugh *et al.*, 1973).

significant difference from histologically normal liver: †p<0.01; *p<0.005; ❖p<0.0005
Figure 4.1

*In vivo* hepatic $^{31}$P MR spectra acquired with TR 5000ms from:

a) a healthy volunteer
b) a patient with compensated cirrhosis and
c) a patient with decompensated cirrhosis

PME = phosphomonoester;
Pi = inorganic phosphate,
PDE = phosphodiester;
PCr = phosphocreatine; $\gamma$, $\alpha$ and $\beta$ATP = ATP.
Figure 4.2

a) *In vivo* hepatic $^{31}$P MR spectrum from a patient with compensated cirrhosis.
b) *In vitro* hepatic $^{31}$P MR spectrum from a perchloric acid extract of liver tissue from the same patient.

MDP = methylene diphosphonate (external reference standard used for quantification of metabolites), NDP = nucleotide diphosphates, NTP = nucleotide triphosphates.
Figure 4.3

In vitro hepatic $^{31}\text{P}$ MR spectrum from a perchloric acid extract of liver tissue from a patient with decompensated cirrhosis. The spectrum has been expanded to show the PME and PDE regions.

PE = phosphoethanolamine, PC = phosphocholine, GPE = glycerophosphorylethanolamine, GPC = glycerophosphorylcholine.
Chapter 5

IN VIVO HEPATIC PHOSPHORUS-31
MR SPECTROSCOPY IN LIVER TRANSPLANT PATIENTS WITH CHRONIC DUCTOPENIC ALLOGRAFT REJECTION
5. HEPATIC PHOSPHORUS-31 MR SPECTROSCOPY IN LIVER TRANSPLANT PATIENTS WITH CHRONIC DUCTOPENIC REJECTION

5.1 Summary

Hepatic phosphorus-31 magnetic resonance spectroscopy (\(^{31}\)P MRS) was undertaken in 16 patients following liver transplantation to assess metabolite changes in allograft rejection. Four individuals had biopsy-proven evidence of chronic ductopenic rejection and 12 had good graft function. The reference population comprised 23 healthy volunteers who had no history, clinical or biochemical evidence of liver disease.

In vivo hepatic \(^{31}\)P MR spectra were acquired with a pulse angle of 45° and repetition times (TR) of 500ms and 5000ms. Peak area ratios of phosphomonoesters (PME), inorganic phosphate (Pi) and phosphodiesters (PDE) relative to \(\beta\)ATP were calculated from spectra acquired at TR 5000ms. Signal height ratios (SHR) were obtained by dividing the peak height at TR 5000ms by that at TR 500ms.

When compared to healthy volunteers, the 12 patients with good graft function displayed no spectral abnormalities; the four patients with chronic ductopenic rejection showed a significantly elevated PME/ATP (\(p<0.01\)), PDE/ATP (\(p<0.005\)) and PME SHR (\(p<0.05\)).

The increase in PME/ATP and PME SHR is probably due to increases in levels of phospholipid precursors, reflecting altered phospholipid metabolism. The elevation in PDE/ATP may reflect a significant contribution from bile in these cholestatic patients.

Hepatic \(^{31}\)P MRS provides information on the pathophysiological mechanisms of liver damage and may be useful in the early detection of chronic graft rejection.
5.2 Introduction

Orthotopic hepatic transplantation (OLT) has become the treatment of choice for end-stage liver failure, but between 2% and 17% of patients subsequently develop chronic graft rejection, occurring most often between six weeks and six months postoperatively (Wiesner et al., 1993). The syndrome is characterised by biliary stasis from the loss of small bile ducts and has been termed ductopenic rejection or the vanishing bile duct syndrome (Wiesner et al., 1991). In the majority of cases this results in irreversible liver damage, often requiring retransplantation (Noack et al., 1991). However, there is some evidence to suggest that altering immunosuppressive regimens, especially to tacrolimus (FK506), may alter the course of chronic ductopenic rejection if the disorder is diagnosed at an early stage (Hubscher et al., 1991; European FK506 Multicentre Liver Study Group, 1994). This emphasises the need for early detection of the condition. Histological examination remains the diagnostic gold standard (Wiesner et al., 1993), but it is subject to sampling error because the liver may be affected unevenly.

Hepatic phosphorus-31 magnetic resonance spectroscopy (\(^{31}\)P MRS) is a non-invasive technique, which may have diagnostic potential in allograft rejection. It provides information on phospholipid membrane metabolism and on the energy status of the liver. A variety of liver diseases have been studied using \(^{31}\)P MRS (Angus et al., 1990; Cox et al., 1992; Munakata et al., 1993), but there is no information on patients with chronic ductopenic rejection.

5.3 Aims

The aims of this study were to delineate abnormalities in the hepatic MR spectra of patients with chronic ductopenic rejection and to compare the spectral appearances with those of patients who had good graft function.
5.4 Subjects and Methods

Twenty-two patients were assessed for MRS study following hepatic transplantation, but six of these individuals failed to complete the spectroscopic examination either because of discomfort or claustrophobia.

The study population therefore comprised 16 patients (six men and 10 women) of mean age 39 (range 22-68) years, all of whom had undergone OLT for chronic liver failure. The mean time interval between transplant surgery and MRS examination was six months (range 10 days - 30 months). All patients had received azathioprine, one patient was on tacrolimus and 15 patients were taking cyclosporin as part of their immunosuppressive regimen. Four individuals had biochemical evidence (Table 5.1) and biopsy-proven evidence of severe ductopenic rejection. The initial indication for transplantation was primary sclerosing cholangitis in three of these individuals and autoimmune chronic active hepatitis in one individual. Three of the patients with ductopenic rejection were subsequently retransplanted because of graft failure. The remaining 12 individuals had good graft function on the basis of standard biochemical liver function tests (Table 5.1) and liver biopsy appearances; the MRS examination taking place after a considerably shorter postoperative period than those subjects with chronic ductopenic rejection (Table 5.1).

The reference population comprised an age matched group of 23 healthy volunteers who had no history, clinical or biochemical evidence of liver disease (mean age 37 [range 25-58] years). None of these individuals was taking regular medication.

All MRS studies were undertaken after an overnight fast. Blood was drawn for standard laboratory liver function tests immediately prior to each MRS examination. Individuals were excluded from the study if they had cardiac pacemakers or other ferromagnetic implants. All subjects provided written, informed consent. Permission for this study was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC/4047) and the Royal Free Hospital and School of Medicine, London.
5.4.1 NMR methods

Hepatic $^{31}$P MR spectra were obtained using a Picker prototype spectroscopy system (Picker International, Cleveland, Ohio), based on a whole body magnet (Oxford Magnet Technology, Oxford, U.K.) operating at 1.5 Tesla. An enveloping transmitter body coil and a separate surface receiver coil were used, both double-tuned for protons at 64 MHz and phosphorus at 26 MHz. The proton signal was used for shimming and to obtain a $T_1$-weighted image in the axial plane to confirm spectral localisation. Localised spectra were obtained from eight parasagittal planes, each of nominal width 30 mm, using a one dimensional chemical shift imaging (1-D CSX) technique (Bailes et al., 1987). Data were collected with repetition times (TR) of 5000 ms (16 averages) and 500 ms (64 averages) with a pulse angle of 45°. The total examination time varied between 45 and 60 minutes.

5.4.2 Data Processing

As in the previous study, the *in vivo* spectra were processed with a cosine filter in the spatial domain and a 60 ms exponential (or 5 Hz Lorentzian) filter in the time domain. A single observer (S.D.T.-R.), who was blinded to the patients' clinical condition, manually phased all spectra in this study. The delay in applying the localising phase-encoding gradients in the 1-D CSX sequence resulted in the appearance of a baseline roll. This artefact was removed using knowledge-based data processing (Saeed et al., 1993). The same observer measured peak area ratios for phosphomonesters (PME), inorganic phosphate (Pi) and phosphodiesters (PDE), relative to $\beta$ATP (ATP) in the baseline corrected spectra. All spectra were fitted to inverse polynomial functions, using the NMR1® spectral processing program (New Methods Research, Inc., E. Syracuse, N.Y.) on a SUN SPARCstation 10 (Sun Microsystems, Inc., Mountain View, CA).
The peak heights of spectral metabolites were used to calculate signal height ratios (SHR) using the formula: \( \text{SHR} = \frac{\text{TR 5000ms}}{\text{TR 500ms}} \). This provided a measure of \( T_1 \) values (see Chapter 1).

5.4.3 Statistical Analysis

The 95% confidence intervals for the individual metabolite ratios in the healthy volunteers were used to define the reference range. Values outside this range were considered abnormal. Non-parametric tests were used for all statistical analyses, because the data were not normally distributed. Metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test. Comparisons between patient groups were made using the Mann Whitney U test with a Bonferroni correction where necessary. Spearman rank correlations were calculated to determine the relationship between the measured biochemical variables and the metabolite ratios. In all cases a \( p \) value of \(<0.05\) was considered to be significant.

5.5 Results

The hepatic \( ^{31}\text{P} \) MR spectra from healthy adult volunteers contained six resonances attributable to PME, Pi, PDE and three peaks which were assigned to \( \gamma \text{ATP}, \alpha \text{ATP} \) and \( \beta \text{ATP} \) (Figure 5.1a). The ATP peak contains contributions from \( \alpha \text{ADP} \) and NADH. The \( \alpha \text{ATP} \) resonance contains a contribution from \( \beta \text{ADP} \). A small resonance arising from phosphocreatine (PCr) was present in some of the spectra, because the MR localisation techniques do not completely exclude signal from the anterior and posterior abdominal wall muscle.
The same resonances were seen in the hepatic $^{31}$P MR spectra from the patient population (Figure 5.1). There was no significant difference in the mean PME/ATP ratio between the reference population and the 12 patients with good graft function (Table 5.2), but four of these patients had a PME/ATP ratio at the upper end of the reference range. The mean PME/ATP ratio was significantly elevated in the four patients with chronic ductopenic rejection compared to the healthy volunteers ($p<0.01$) (Table 5.2), but not compared to patients with good graft function (Table 5.2).

There was no significant difference in PME signal height ratio (SHR) between the 12 patients with good graft function and the 23 healthy volunteers. However, the mean PME SHR was significantly elevated in patients with chronic ductopenic rejection compared to both the reference population ($p<0.01$) and the patients with good graft function ($p<0.05$) (Table 5.3).

There were no statistically significant differences in the Pi/ATP ratio or the Pi SHR between the patient and reference populations. Furthermore, the PDE/ATP ratio for the 12 patients with good graft function was not significantly different from that for the reference population (Table 5.2), but the four patients with chronic ductopenic rejection all showed a significant elevation in the PDE/ATP ratio compared to both healthy volunteers ($p<0.005$) and patients with good graft function ($p<0.005$) (Table 5.2).

There was no significant difference in PDE SHR between the patient and reference populations, but there was a significant direct correlation between the PDE/ATP ratio and serum bilirubin ($p<0.0001$) and serum alkaline phosphatase levels ($p<0.0005$).
5.6 Discussion

Following orthotopic liver transplantation, allograft rejection is a major cause of morbidity and of late graft failure. The incidence of acute cellular rejection, developing usually in the first two weeks post-transplantation, is between 50% and 100% (Wiesner et al., 1993). This may be overcome by manipulation of anti-rejection chemotherapy, including the administration of high dose corticosteroids (Wiesner et al., 1993). The immunological processes occurring in the later post-operative period are characterised by obliterative vasculopathy and the progressive loss of interlobular and septal bile ducts (Rubin and Munoz, 1993). This chronic ductopenic form of rejection affects on average 8% of transplant patients (Wiesner et al., 1993) and results in severe cholestasis. It is usually associated with progressive graft dysfunction and may require retransplantation (Noack et al., 1991).

In this study, patients with chronic ductopenic rejection showed significant elevations of the mean PME/ATP ratio, mean PME SHR and the mean PDE/ATP ratio when spectra were compared to those from the reference population. The $^{31}$P MR spectra obtained from patients with good graft function were indistinguishable from those from healthy volunteers.

It is true that patients with ductopenic rejection were examined after a considerably longer post-operative interval than the patients with good graft function. The four patients with chronic graft failure were recruited for MRS study when they were being considered for retransplantation but, unfortunately, it was not possible to recruit patients with well functioning grafts after this extended time period. Similarly, no patients were examined prior to the 10th post-operative day, because the use of metallic wound clips rendered MRS examination impossible.
Signal height ratio (SHR) values determined from data acquired at TR 500ms and 5000ms provide an estimate of changes in $T_1$, which is the MR time constant governing the recovery of longitudinal magnetization after excitation. Different metabolites have different $T_1$ values and these may change with disease. In the present study, the mean PME SHR in patients with chronic ductopenic rejection was significantly increased, compared to that of both healthy volunteers and patients with good graft function, although the Pi SHR and PDE SHR remained unaltered. Both the PME and the PDE resonances are multicomponent and the constituents are not resolved at the magnetic field strengths employed in human in vivo $^{31}$P MRS studies. The $T_1$ changes indicated by the differences in SHR probably arise from a variation in the relative contribution of different components to the PME resonance. In order to fully separate the components of the in vivo MR peaks, it is necessary to undertake in vitro $^{31}$P MRS studies of liver tissue at higher magnetic field strengths (Bell et al., 1993).

The PME peak includes contributions from phosphocholine (PC) and phosphoethanolamine (PE), intermediates on the pathway of phospholipid membrane synthesis (Ruiz-Cabello and Cohen, 1992), as well as contributions from adenosine monophosphate and glycolytic intermediates (sugar phosphates) (Bell et al., 1993). The elevation of the PME/ATP ratio in patients with chronic ductopenic rejection may represent an increased contribution of PE and PC to the PME resonance. These metabolites have been shown to increase during hepatic regeneration in animal studies (Murphy et al., 1992) and it would seem likely that in conditions of rapid cell turnover, such as hepatic graft failure, these cell membrane precursors are increased.

The PDE resonance is also composite, including signal from glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), intermediates on the pathway of phospholipid breakdown (Ruiz-Cabello and Cohen, 1992) and a contribution from
endoplasmic reticulum (Murphy et al., 1989). The increase in the PDE/ATP ratio which was observed in the individuals with chronic ductopenic rejection may be due to a larger contribution of cell membrane breakdown products to the PDE resonance (Ruiz-Cabello and Cohen, 1992), but in preliminary in vitro hepatic $^{31}$P MRS studies, GPE and GPC do not appear to be elevated in liver biopsy samples from patients with this condition (Bell JD, personal communication).

Similarly, a compensatory increase in hepatocyte endoplasmic reticulum (Murphy et al., 1989) could be responsible for the observed elevation in the PDE/ATP ratio from these patients with poor graft function. This hypothesis may be further investigated using electron microscopy and in vitro analysis of lipid extracts of liver samples, obtained from the chronically rejecting liver at retransplantation.

Chronic ductopenic rejection is characterised by interlobular and septal bile duct loss (Wiesner et al., 1993) and one further explanation of the elevation in the PDE/ATP ratio may be a significant contribution from bile in these severely cholestatic patients. Such spectral abnormalities may not be specific to this syndrome, because patients with other cholestatic conditions, such as primary biliary cirrhosis and primary sclerosing cholangitis, also have a higher PDE/ATP ratio than may be expected (Jalan et al., 1995). However, such changes in the PDE/ATP ratio were of a smaller order of magnitude to those which were observed in individuals with chronic ductopenic rejection.

Although there may be other histological abnormalities in patients with late hepatic dysfunction, ductopenia is the salient feature of chronic rejection and where the bile duct loss is progressive and inexorable, this has been termed the vanishing bile duct syndrome (Wiesner et al., 1991). The diagnosis of this condition can be difficult and rests to a large extent on histological examination of liver biopsy samples (Rubin and Munoz, 1993). Typically, there
is loss of bile ducts in at least 50% of the portal tracts (Wiesner et al., 1993). However, the condition affects the liver patchily and the diagnosis often may only be made on comparison of serial biopsies (Rubin and Munoz, 1993).

There is evidence to suggest that the vanishing bile syndrome may be reversible in some individuals (Hubscher et al., 1991; European FK506 Multicentre Liver Study Group, 1994), consequent to manipulation of anti-rejection chemotherapy. Therefore, a reliable means of early diagnosis is required. Hepatic $^{31}$P MRS is a non-invasive technique and can provide information from large areas of the liver. The findings of both an elevated PME/ATP ratio and PDE/ATP ratio in patients with chronic ductopenic rejection are novel and in situations where liver biopsy has yielded an inconclusive result, may possibly be of use in diagnosis of patients with this condition.

Further studies are needed to determine the pathogenic mechanisms underlying our MRS findings. Such studies must involve a correlation between $in$ $vivo$ $^{31}$P MRS, $in$ $vivo$ $^{31}$P MRS analysis of liver biopsy material, electron microscopy of such samples and $in$ $vitro$ analysis of lipid extracts of liver tissue.
5.7 References


110
Table 5.1  Liver function tests of study patients

mean (range) values

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Interval since transplant (days)</th>
<th>Serum bilirubin (μmol/L) (5-17)*</th>
<th>Serum aspartate transaminase (u/L) (5-40)*</th>
<th>Serum alkaline phosphatase (u/L) (35-130)*</th>
<th>Plasma albumin (g/L) (35-50)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good graft function</td>
<td>56 (10-249)</td>
<td>29 (14-59)</td>
<td>26 (12-42)</td>
<td>150 (89-232)</td>
<td>38 (34-47)</td>
</tr>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>492 (280-851)</td>
<td>323 (263-419)</td>
<td>272 (150-594)</td>
<td>855 (260-1660)</td>
<td>30 (27-34)</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* reference range
Table 5.2 Metabolite ratios in the patient and reference populations

mean (±1 SD) values

<table>
<thead>
<tr>
<th>Study group (n)</th>
<th>PME/ATP</th>
<th>Pi/ATP</th>
<th>PDE/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers (n=23)</td>
<td>0.77 ± 0.20</td>
<td>0.85 ± 0.26</td>
<td>3.30 ± 0.55</td>
</tr>
<tr>
<td>Good graft function (n=12)</td>
<td>0.89 ± 0.24</td>
<td>0.78 ± 0.24</td>
<td>3.15 ± 0.58</td>
</tr>
<tr>
<td>Chronic rejection (n=4)</td>
<td>1.11 ± 0.14**</td>
<td>0.79 ± 0.27</td>
<td>4.95 ± 0.78*** #</td>
</tr>
</tbody>
</table>

significant difference from healthy volunteers: * p<0.05; ** p<0.01; *** p<0.005
significant difference from patients with good graft function: # p<0.005

Table 5.3 Signal height ratios (SHR) in patient and reference populations. mean (±1 SD) values

<table>
<thead>
<tr>
<th>Study group (n)</th>
<th>PME SHR</th>
<th>PDE SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers (n=23)</td>
<td>1.93±0.51</td>
<td>2.59±0.60</td>
</tr>
<tr>
<td>Good graft function (n=12)</td>
<td>2.13±0.61</td>
<td>2.44±0.68</td>
</tr>
<tr>
<td>Chronic rejection (n=4)</td>
<td>3.95±1.13** #</td>
<td>2.80±1.12</td>
</tr>
</tbody>
</table>

significant difference from healthy volunteers: * p<0.05; ** p<0.01
significant difference from patients with good graft function: # p<0.05
Figure 5.1

Hepatic $^{31}$P MR spectra from:

a) a healthy volunteer  
b) a patient with good graft function and  
c) a patient with chronic ductopenic rejection.

It can be seen that the PME/ATP and PDE/ATP ratios are elevated in the spectrum from the patient with chronic rejection.

PME = phosphomonoester, Pi = inorganic phosphate, PDE = phosphodiester, PCr = phosphocreatine, $\gamma$, $\alpha$, $\beta$ATP = adenosine triphosphate.
* Phosphocreatine
AN INTRODUCTION TO HEPATIC ENCEPHALOPATHY

AND

THE APPLICATION OF MAGNETIC RESONANCE TO PATIENTS WITH THIS CONDITION
6. AN INTRODUCTION TO HEPATIC ENCEPHALOPATHY AND THE APPLICATION OF MAGNETIC RESONANCE TO PATIENTS WITH THIS CONDITION

6.1 Introduction

Hepatic encephalopathy occurs in two distinct forms:

1. As a result of acute liver failure.

   This condition progresses rapidly, over a period of days or even hours and has a high associated mortality if left untreated (Messner, 1992). The predominant neuropathological picture is of cerebral oedema, due to increased permeability of the blood brain barrier (Butterworth, 1995). Death frequently results from cerebral coning with herniation of the brain through the basilar foramena, as a result of increased intracranial pressure from the development of cerebral oedema.

2. As a result of surgical or spontaneous portasystemic shunting in chronic liver disease.

   Portal-systemic encephalopathy (PSE) or chronic hepatic encephalopathy (CHE) are the terms used to describe the neuropsychiatric abnormalities affecting patients with chronic liver disease (Ferenci, 1991). This condition results from either the development of a portasystemic collateral circulation in patients with chronic liver disease, complicated by portal hypertension or from the surgical placement of a portasystemic shunt, usually for the management of uncontrolled variceal bleeding (Fischer, 1992). Such shunts may be performed by standard surgical techniques or else by using transjugular intrahepatic portasystemic stent shunts (TIPSS), which are placed under radiological guidance.
6.2 **The Clinical Spectrum of Chronic Hepatic Encephalopathy**

In the majority of patients, chronic hepatic encephalopathy is subclinical, affecting reaction times in activities of daily living such as driving and operating machinery (Barbara, *et al*., 1992). It is usually detected in slowing of the EEG frequency or as impairment of psychometric performance (Sherlock and Dooley, 1993). However in about 30% of patients with chronic liver disease, the syndrome may become clinically overt with alterations in behaviour, mood, personality and disturbances in consciousness (Conn, 1992). The condition is usually characterised by remissions and relapses, although in some patients the neuropsychiatric impairment may follow a more persistent course. The West Haven mental state grading system, which is the commonly used clinical index for assessing the severity of encephalopathy is detailed in Table 6.1. (Conn *et al*., 1977).

Table 6.1 **West Haven Criteria for grading mental state**

<table>
<thead>
<tr>
<th>Grade 0</th>
<th>No abnormality detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Trivial lack of awareness, euphoria, anxiety. Shortened attention span. Impaired performance in addition or subtraction.</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Lethargy, apathy, disorientation for time and place. Obvious personality change. Inappropriate behaviour.</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Somnolence to semi-stupor, but responsive to stimuli. Confusion. Gross disorientation.</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Coma Mental state not testable</td>
</tr>
</tbody>
</table>

* (Conn *et al*., 1977)
The *de novo* development or clinical worsening of chronic hepatic encephalopathy is usually associated with a precipitating factor such as a gastrointestinal bleed, constipation, occult infections including spontaneous bacterial peritonitis, the adherence to a high animal protein containing diet, electrolyte disturbances following overdiuresis during treatment for ascites or the administration of sedative drugs such as benzodiazepines or opioids (Sherlock and Dooley, 1993).

### 6.3 The Pathogenesis of Hepatic Encephalopathy

The pathogenesis of chronic hepatic encephalopathy is unknown, but it is thought to primarily represent a neurochemical/neurophysiological disorder, rather than a structural disorder of the brain (Zeneroli, 1992). Interest has been focused on the potential pathogenic role of circulating toxins and on changes in the functional state of cerebral neurotransmitter systems. As the syndrome has such a wide clinical spectrum, it is likely that a number of abnormalities occur which act interdependently and possibly synergistically (Butterworth, 1995).

Unlike acute hepatic encephalopathy, where there may be marked cerebral oedema, the most prominent neuropathological feature in the brains of patients with chronic hepatic encephalopathy is a change in the astrocyte population, known as Type II Alzheimer’s astrocytosis (Norenberg, 1987). These cells occur throughout the brain in this patient group, particularly in the basal ganglia and in the cortex, playing an important role in the detoxification of cerebral ammonia (Butterworth *et al.*, 1987).
6.3.1 The Role of Ammonia

Blood ammonia concentrations are often, but not invariably increased in patients with chronic hepatic encephalopathy. Owing to impaired urea synthesis and portasystemic shunting in patients with chronic liver disease, nitrogenous products including ammonia, formed by the action of gut bacteria on dietary protein, reach the brain and subject it to an altered biochemical environment. Disturbances in cerebral ammonia metabolism, together with changes in glutamine and glutamate levels are considered to be of prime pathogenic importance (Butterworth et al., 1987).

Glutamate is the major excitatory neurotransmitter in the brain and it is reduced in patients with chronic hepatic encephalopathy (Butterworth et al., 1987). Regulation at the glutamate synapse involves a functional interaction between the presynaptic and postsynaptic neurones, as well as the perineuronal astrocytes. There is evidence to suggest that glutamate synaptic regulation is impaired in patients with chronic hepatic encephalopathy (Butterworth, 1992).

Glutamine, an amino acid formed by amidation of glutamate, plays an important role in ammonia detoxification in the brain, but may itself be neurotoxic. Cerebral glutamine levels are considerably elevated in hyperammonaemic states such as those seen in this patient population. This is essentially because the excess cerebral ammonia is converted to glutamine by the action of the enzyme glutamine synthetase on glutamate. The site of this reaction is within the astrocytes and this enzyme is particularly active in the abnormal population of Alzheimer II cells, which are present in patients with hepatic encephalopathy (Butterworth et al., 1987).

Ammonia itself may have a direct effect on neurotransmission, adversely affecting presynaptic (Raabe, 1987) and postsynaptic potentials, reducing the neuronal release of glutamate (Fan et al., 1990) and reducing its reuptake and reutilisation by astrocytes (Butterworth, 1992).
6.3.2 Impaired Energy Metabolism

Brain glucose and oxygen consumption are both significantly reduced in patients with chronic hepatic encephalopathy (Butterworth, 1995). Reduced brain glucose metabolism in early encephalopathy is probably the result of decreased energy demands rather than the primary cause of neurological dysfunction. Animal studies suggest that high energy phosphates are preserved until the encephalopathy is advanced (Hindfelt et al., 1977).

6.3.3 Other Neurotransmitter Pathways

It is unlikely that ammonia toxicity is the sole cause of hepatic encephalopathy. Other circulating gut derived toxins such as mercaptans, short-chain fatty acids, GABA and endogenous benzodiazepines have all been implicated in the neurotransmitter imbalance, which tips the balance towards neuroinhibition and the clinical manifestations of hepatic encephalopathy (Ferenci, 1991). Similarly, the reduction of circulating branched chain amino acids in patients with chronic liver disease and the relative increase in aromatic amino acids such as tryptophan, favours the production of serotonin, quinolinic acid and tryptamine. All of these tryptophan metabolites are neuroactive and may be neurotoxic in encephalopathic patients (Jonung et al., 1983).

6.4 The Investigation of Hepatic Encephalopathy

Difficulties arise in the study of hepatic encephalopathy for a number of reasons. First it has been difficult to study cerebral metabolic function in man non-invasively; second, the animal models of hepatic encephalopathy currently available are not entirely satisfactory and third, results based on in vitro studies or on analysis of post-mortem brain samples are difficult to extrapolate to the in vivo situation. In addition, uncertainty often exists as to the causal relationship, if any, between the abnormalities observed and the clinical findings.
In vivo nuclear magnetic resonance spectroscopy (MRS) is a non-invasive technique which can be used to provide localised biochemical information on cerebral metabolic processes and which might usefully be applied to the study of hepatic encephalopathy.

A typical $^1$H MR spectrum of the brain contains choline (Cho), creatine (Cr), glutamine/glutamate (Glx) and N-acetyl aspartate resonances (Ross et al., 1992). Ammonia cannot be detected by $^1$H MRS because of rapid proton exchange with water, but changes observed in the Glx resonance reflect ammonia incorporation into glutamine (Chamuleau et al., 1991).

Cerebral oxygen consumption and cerebral glucose utilisation studies using FDG-glucose for PET show patients with chronic hepatic encephalopathy have a significant reduction in brain energy metabolism (Butterworth et al., 1995). Similarly, $^{31}$P MRS gives information on high energy phosphates and on glycolytic intermediates in the brain, but in a non-invasive fashion. It too may be applied to the study of patients with this condition.

6.5 Treatment and Patient Monitoring

There is no 'specific' treatment for chronic hepatic encephalopathy, but there are a number of non-specific measures which can be used singly or in combination (Bircher, 1992). The main aim is to reduce the circulating ammonia levels, either by reducing the gut bacteria load through bowel sterilisation with agents such as neomycin or the promotion of a regular bowel movement, greater than twice per day, with non-absorbable disaccharides such as lactitol or lactulose. The urea cycle can be promoted with the use of ornithine salts, thus metabolising ammonia appropriately in the failing liver. However, more targeted treatment such as benzodiazepine antagonists or branched chain amino acids to redress the circulating amino acid imbalance in patients with cirrhosis have not met with universal usefulness (Ferenci, 1991).
The natural history of hepatic encephalopathy is unknown. Therefore it is not known whether the patients who are mentally normal will remain so, or whether patients with subclinical encephalopathy will progress to the overt forms of the disease and if so, at what rate. One of the standard means of assessing hepatic encephalopathy is psychometry, which may be subject to a learning curve and thus it may not be an entirely objective means of assessment, while electroencephalography and blood ammonia levels do not necessarily correlate with the severity of neuropsychiatric impairment (Conn, 1992).

In animals with encephalopathy, inhibition of cerebral glutamine synthetase and hence reduction in cerebral glutamine concentrations, results in an improvement in the condition (Hawkins et al., 1993). Similarly in man, treatment of chronic hepatic encephalopathy is aimed at reducing circulating ammonia concentrations and hence by extrapolation cerebral glutamine concentrations. Magnetic resonance spectroscopy (MRS) allows the non-invasive study of cerebral metabolism in vivo. This technique can be used to provide localised biochemical information on cerebral metabolic processes and might usefully be applied to the study of hepatic encephalopathy. It may prove to be useful in disease monitoring and in gauging response to treatment, since the use of proton MR spectroscopy ($^1$H MRS) allows glutamine levels to be monitored directly in the human brain in vivo.
6.6 PHOSPHORUS-31 MAGNETIC RESONANCE SPECTROSCOPY

Phosphorus-31 MRS provides information on cerebral phospholipids, sugar phosphates, and high energy phosphates such as phosphocreatine and ATP (Coutts et al., 1989). The cerebral $^{31}$P MR spectrum from a healthy volunteer contains at least seven resonances (Bottomley, 1984) which can be assigned to phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr), ATP, ATP and $\beta$ATP. The PME and PDE peaks are multicomponent, the ATP peak contains contributions from $\alpha$ADP and NADH and the ATP contains contributions from $\beta$ADP. This technique affords the possibility of following cellular bioenergetics non-invasively in patients with chronic hepatic encephalopathy.

Some phosphorus-31 MR studies of the brain have been previously undertaken in patients with chronic hepatic encephalopathy, but there has been no consensus of opinion as to the spectral abnormalities found in such patients. Correlation with the severity of neuropsychiatric impairment was also lacking in these studies (Luyten et al., 1989; Barbiroli et al., 1992; Barbara et al., 1993).

6.7 PROTON MAGNETIC RESONANCE SPECTROSCOPY

Proton MRS can be utilised to provide information on brain metabolites such as choline, creatine, N-acetyl aspartate (NAA), myoinositol, glutamine and glutamate (Ross et al., 1992). A typical proton MR spectrum of the brain contains resonances which can be assigned to the methyl moieties of choline (Cho) at 3.22 ppm, creatine (Cr) at 3.02 ppm and NAA at 2.02 ppm. The region of the proton MR spectrum between 2.1 and 2.5 ppm includes contributions from both glutamine and glutamate (Kreis et al., 1991).
A number of proton MRS studies of the brain have been undertaken in patients with chronic hepatic encephalopathy. These show a reduction in the choline and myoinositol resonances and an increase in the composite glutamine/glutamate resonances in the cerebral cortex (Kreis et al., 1991). However, no attempts to correlate spectroscopic changes with electroencephalography, psychometry and blood ammonia levels have been made. Furthermore, only information from the cerebral cortex has been reported with no studies having been focussed on other areas of the brain such as the basal ganglia, which are abnormal on MR imaging of this patient population.

6.8 MAGNETIC RESONANCE IMAGING

T₁-weighted magnetic resonance imaging (MRI) studies of the brain in patients with chronic liver disease have demonstrated hyperintensity in the basal ganglia. This observation was first reported by Brunberg and colleagues (Brunberg et al., 1991). The underlying cause of these abnormalities and the clinical parameters which correlate with the increase in signal intensity in the basal ganglia of such patients are still unclear. While magnetisation transfer imaging highlights the basal ganglia, no formal study has been performed in patients with chronic hepatic encephalopathy.
6.9 AIMS

The aims of this study were fourfold:

1. To assess changes in cerebral $^{31}$P MR spectra in patients with cirrhosis and to relate any changes observed to the patients' neuropsychiatric status.

   *(The results of this study are detailed in Chapter 7)*

2. To assess regional variations in proton MR spectra in the brains of patients with biopsy-proven cirrhosis and relate any changes observed to the patients' neuropsychiatric status.

   *(The results of this study are detailed in Chapter 8)*

3. To measure the changes observed on T$_1$-weighted and magnetisation transfer MR imaging of the basal ganglia in chronic liver disease and to correlate the quantified signal intensities with the clinical indices of the patients' neuropsychiatric status and the functional severity of their liver disease.

   *(The results of this study are detailed in Chapter 9)*

4. To correlate the hyperintensity of the globus pallidus on T$_1$-weighted MR imaging of the basal ganglia in chronic liver disease with the MR spectroscopy abnormalities measured in both proton and phosphorus-31 MR spectra localised to the same region of the brain.

   *(The results of this study are detailed in Chapter 10)*
6.10 References


Chapter 7

CEREBRAL PHOSPHORUS-31
MAGNETIC RESONANCE SPECTROSCOPY
IN PATIENTS WITH
CHRONIC HEPATIC ENCEPHALOPATHY
7. CEREBRALPHOSPHORUS-31 MAGNETIC RESONANCE SPECTROSCOPY IN PATIENTS WITH CHRONIC HEPATIC ENCEPHALOPATHY

7.1 Summary

Cerebral phosphorus-31 magnetic resonance spectroscopy (\(^{31}\)P MRS) was undertaken in 33 patients with biopsy-proven cirrhosis; six had no evidence of neuropsychiatric impairment on standard clinical, psychometric and electrophysiological testing; eight had evidence of subclinical hepatic encephalopathy and 19 were classified as having overt hepatic encephalopathy. The reference population comprised 15 healthy volunteers.

Unlocalised spectra were acquired from the entire head with a 45° pulse angle and repetition times (TR) of 1000ms and 5000ms. Spectra localised to the basal ganglia were acquired with a 45° pulse angle and a TR of 1000ms. Peak area ratios of phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE) and phosphocreatine (PCr) relative to βATP (ATP) were measured in the spectra acquired.

There was no consistent change in Pi/ATP and PCr/ATP. Mean values of PME/ATP and PDE/ATP were significantly lower in the total patient population than in the reference population and correlated with the patients' neuropsychiatric status. Thus, there were no significant reductions in mean PME/ATP and mean PDE/ATP in patients who were neuropsychiatrically unimpaired, but significant reductions were observed in mean PME/ATP and mean PDE/ATP in patients with both subclinical and overt hepatic encephalopathy. The most marked reductions in these metabolite ratios were observed in patients with overt encephalopathy and in two of these patients, treatment with lactitol resulted in increases in the PME/ATP and PDE/ATP, which were most obvious in the localised spectra.
The objective changes in $^3$P MR spectra with the severity of chronic hepatic encephalopathy suggest that cerebral $^3$P MRS could be used to diagnose the presence of hepatic encephalopathy and to monitor treatment effects.

7.2 Introduction

The monitoring of patients with chronic hepatic encephalopathy and their response to different treatment modalities rests to a large extent on clinical examination and the performance of serial psychometric testing. However, both methods of patient assessment may be subjective and in patients with subclinical disease, who are outwardly normal, difficulties may arise in adequately following their progress. Electroencephalography has been used in to study patients with chronic liver disease, but the characteristic slowing of the mean cycle frequency to below 8.9 cycles per second does not necessarily correlate with disease severity (Schomerus et al., 1981).

In vivo nuclear magnetic resonance spectroscopy (MRS) is a non-invasive technique which can be used to provide localised biochemical information on cerebral metabolic processes and which might usefully be applied to the study of the pathogenesis of hepatic encephalopathy and may find use in the monitoring of such patients.

A number of cerebral $^3$P MR studies have been undertaken in patients with chronic hepatic encephalopathy (Ross et al., 1987; Luyten PR et al., 1989; Chamuleau et al., 1991b; Barbiroli et al., 1992; Barbara et al., 1993), but there has been no real consensus of opinion as to the cerebral $^3$P MRS findings in this patient group. Such studies have studied only small numbers of patients and no attempt was made to relate MR spectroscopy findings to the severity of hepatic encephalopathy in the study populations.
7.3 Aims

The aims of the present study were to assess changes in cerebral $^{31}$P MR spectra in patients with cirrhosis and to relate any changes observed to the patients' neuropsychiatric status.

7.4 Subjects and Methods

The patient population comprised 33 individuals (18 men and 15 women) with biopsy-proven cirrhosis of mean (range) age 52.2 (32-67) years. Twenty three patients (69.7%) had alcohol related liver disease, five (15.2%) cryptogenic cirrhosis, three (9.0%) biliary cirrhosis and two (6.1%) autoimmune chronic active hepatitis/cirrhosis. Functionally four (12.1%) were Child's grade A, 20 (60.6%) Child's grade B and nine (27.3%) Child's grade C (Pugh et al., 1973). All patients had been abstinent from alcohol for a minimum of 3 months and none was on psychoactive medication.

The reference population comprised 15 healthy volunteers (seven men and eight women) of mean (range) age 40.8 (21-67) years; none drank alcohol in excess of 20 g/day and none was taking regular medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant.

All individuals studied provided written informed consent. Permission for this study was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC 93/4047) and the Royal Free Hospital and School of Medicine, London.

7.4.1 Patient Assessment

All patients underwent full neurological, psychometric and electrophysiological assessment on the morning of MRS study. Patients were examined clinically and their mental state
assessed using West Haven criteria (Conn et al., 1977). Psychometric performance was assessed under standardised conditions and by the same observer, using a battery of four tests comprising Number Connection Tests (NCT) A and B (Conn, 1977), the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). Electroencephalograms (EEG) were performed using conventionally placed electrodes and a mean cycle frequency was obtained. The blood ammonia concentration was measured in each subject immediately prior to MRS examination using a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co, Ltd, Kyoto, Japan) (Quero et al., 1993). A PSE sum (Conn, 1977) was then calculated for each subject.

The patient population was classified into three groups on the basis of this assessment (Table 7.1). Six individuals had no history of hepatic encephalopathy and showed no neuropsychiatric abnormalities when assessed; they were classified as being neuropsychiatrically unimpaired.

Eight individuals had no history of overt hepatic encephalopathy, were clinically normal on examination, but showed slowing of their EEG mean cycle frequency to below the reference range for an alert adult of ≥8.9 cycles per second (cps) and/or impaired performance in at least two of the four psychometric tests employed. These patients were classified as having subclinical hepatic encephalopathy; three patients in this group were on maintenance treatment with lactitol (Morgan et al., 1989).

The remaining 19 patients either had overt untreated hepatic encephalopathy (n=2) or else gave a history of overt hepatic encephalopathy requiring long-term maintenance treatment with the non-absorbable disaccharides, lactulose or lactitol (n=17) (Morgan et al., 1989).
The two patients with untreated overt hepatic encephalopathy were re-examined after eight weeks of treatment with lactitol.

7.4.2 NMR Methods

In vivo cerebral $^{31}$P MR spectra were obtained using a Picker prototype spectroscopy system, based on a whole body magnet (Oxford Magnet Technology, Oxford, U.K.), operating at 1.5 Tesla (Coutts et al., 1989). An enveloping saddle-shaped transmitter coil and a separate saddle-shaped receiver coil, into which each subject’s head could be comfortably positioned, were employed for all examinations. Both coils were double-tuned for phosphorus and proton frequencies at 25.9 MHz and 64 MHz respectively. The proton signal was used for shimming and to acquire $T_1$-weighted axial imaging to verify spectral localisation.

Unlocalised spectra were acquired from the entire head in all 48 subjects, using repetition times (TR) of 1000ms and 5000ms. Data were accumulated for 128 averages at 1000ms and 64 averages at 5000ms; each examination took 2 minutes and 5 minutes respectively.

Localisation was achieved employing a three dimensional chemical shift imaging (3-D CSI) technique (Coutts et al., 1989). A total of 512 voxels in a cubic array, 8x8x8 in dimensions, covering the whole head was acquired, with each voxel containing a conventional MR absorption spectrum. A total of 2048 averages were acquired for the 3D-CSI examination at a TR of 1000ms; a nominal spatial resolution of $(3\text{cm})^3$ was obtained in approximately 34 minutes.

For both unlocalised and localised examinations a 45° radiofrequency (rf) pulse was employed. The pulse was calibrated using an external pick-up loop, which monitored the rf field directly. The variations in loading upon the transmitter system between phantom
calibration studies and *in vivo* human studies were corrected for by this procedure. The choice of a 45° rf pulse is commensurate with improving spectral appearances or signal-to-noise ratio (SNR) in instances where TR is sufficiently low that resonances are partially saturated (Ernst and Anderson, 1966). The full protocol for this study, as described above, took approximately 120 minutes.

### 7.4.3 Data Processing

The unlocalised spectra were processed with an exponential difference filter of 1 and 60 milliseconds (ms) and were manually phased. The 1 ms exponential filter was employed to remove the broader resonance which is usually assigned to bone and lipid components. SNR was improved by line broadening with the 60 ms exponential (or 5 Hz Lorentzian) filter. The localised spectra were processed with a cosine filter in all three spatial directions, a 30 ms exponential filter and were also manually phased. The filtering of the broad resonance was unnecessary because of the introduction of the spatial localising field gradient pulses. The baseline roll, resulting from this delay in data acquisition while phase-encoding gradients were applied in the 3-D CSI sequences, was removed using a knowledge-based algorithm (Saeed and Menon, 1993). A manual baseline correction was used, where necessary, and peak areas ratios of PME, Pi, PDE and PCr relative to $\beta$ATP and PCr/Pi were measured by a blinded observer, using the NMRl® spectral processing program (New Methods Research, Inc, E. Syracuse, N.Y.) on a SUN SPARCstation 1+ (Sun Microsystems, Inc, Mountain View, C.A.). The data were fitted to inverse polynomial functions.

### 7.4.4 Statistical Analysis

The 95% confidence intervals for the individual metabolite ratios in the healthy volunteers were used to define the reference range. Values outside this range were considered abnormal. Since the data were not normally distributed, non-parametric tests were used for
all statistical analyses. Values for the metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test. Comparisons between the patient subgroups were assessed using a non-parametric analysis of variance (Kruskal-Wallis). Comparisons between individual patient subgroups and the reference population were made using the Mann Whitney U test with a Bonferroni correction, where appropriate. In all cases a p value of <0.05 was considered significant.

7.5 Results

The cerebral $^{31}$P MR spectrum from a healthy volunteer contains at least seven resonances (Bottomley, 1984) which can be assigned to phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiester (PDE), phosphocreatine (PCr), ATP, $\beta$ATP and $\beta$P. The PME and PDE peaks are multicomponent, the ATP peak contains contributions from $\alpha$ADP and NADH and the ATP contains contributions from $\beta$ADP.

Unlocalised spectra were obtained from all subjects. The protocol was not completed by 10 patients and two volunteers owing to subject movement or discomfort and therefore localised spectra were obtained in 23 patients and 13 volunteers.

Spectra localised to the basal ganglia were chosen to be analysed, because several of this patient cohort showed some minor extrapyramidal signs of Parkinsonian type and because hyperintensity of the globus pallidus has been observed on $T_1$-weighted images in a high percentage of patients with chronic liver disease (Kulisevsky et al., 1992). Only the unlocalised spectra and those spectra localised to the basal ganglia were used for further comparisons.

There were no additional resonances noted in the patients' spectra (Figures 7.2 and 7.3), but there were significant changes in certain metabolite ratios. The mean ($\pm$1SD) PME/ATP was significantly reduced in the patient population, compared to the reference population, in both
unlocalised spectra and in spectra localised to the basal ganglia (Table 7.2). Similarly, significant reductions were observed in mean PDE/ATP in the patient population in both unlocalised and localised spectra, although the magnitude of the reduction was smaller (Table 7.2).

The changes in mean PME/ATP and mean PDE/ATP correlated overall with the patients' neuropsychiatric status (Table 7.3). No significant reductions in mean PME/ATP and mean PDE/ATP were observed in the patients who were neuropsychiatrically unimpaired, but significant reductions in the mean values for these metabolite ratios were observed in the presence of neuropsychiatric impairment.

Significant reductions in mean PME/ATP were observed in patients with subclinical encephalopathy in both unlocalised and localised spectra (Table 7.3). No significant difference was observed in the mean PDE/ATP in unlocalised spectra acquired with TR 5000ms in these patients, although significant reductions were observed in the mean PDE/ATP in unlocalised spectra acquired with TR 1000ms and in spectra localised to the basal ganglia (Table 7.3).

In the patients with overt hepatic encephalopathy significant reductions were observed in mean PME/ATP and mean PDE/ATP in both the unlocalised spectra, whether acquired with TR 1000ms or 5000ms, and the localised spectra (Table 7.3). The changes in mean PME/ATP and mean PDE/ATP distinguished the patients with overt hepatic encephalopathy from those who were neuropsychiatrically unimpaired (Table 7.3). However, there were no significant differences in mean values for these metabolite ratios between the patients with subclinical hepatic encephalopathy and patients in the other two groups.

There were no significant correlations between these metabolite ratios and the patients' liver function tests, Child's grading, EEG mean cycle frequency, psychometric performance, blood ammonia concentrations or the PSE sum.
In the two patients with untreated overt hepatic encephalopathy, initial spectra showed reduced values for PME/ATP and PDE/ATP (Table 7.4). Treatment with lactitol resulted in increases in these metabolite ratios towards the reference range and were most obvious in the spectra localised to the basal ganglia (Table 7.4). These changes mirrored the improvements observed in the patients' mental state, EEG mean cycle frequency, psychometric performance and blood ammonia concentrations.

There were no consistent changes in mean PCr/ATP or mean Pi/ATP in the total patient population (Table 7.2) and there were no changes in these metabolite ratios which could be related to the patients' neuropsychiatric status (Table 7.3).

7.6 Discussion

In this study of patients with chronic hepatic encephalopathy, there were significant reductions in PME/ATP and PDE/ATP in the cerebral $^{31}$P MR spectra from the total patient population when compared with cerebral spectra from healthy volunteers. These reductions were correlated with the patients' neuropsychiatric status. It is possible that greater differences would have been observed between the patients with the subclinical and overt forms of the syndrome if all the patients had been studied in the untreated state or with more florid clinical signs. However, untreated patients are not often encountered and when the condition is severe MRS examinations may not be feasible.

Differences between the patient and reference populations were often greater in the unlocalised spectra than those localised to the basal ganglia. This may, in part, reflect the lower SNR in the localised spectra (Figure 7.2). This in turn, is a reflection of the smaller volume from which these spectra were obtained, compared to the unlocalised spectra from the entire head. The time for data acquisition was determined by the small voxel size, irrespective of whether a single voxel or multivoxel voxel technique was used. The use of
a 3-D CSI technique allowed the freedom to look at areas of the brain other than the basal ganglia, if necessary, without \textit{a priori} selecting the region of interest and did not prolong the examination time for this study. Few cerebral $^{31}$P MR studies have been undertaken in patients with chronic liver disease and there is little consensus in the published findings (Ross \textit{et al}., 1987; Chamuleau \textit{et al}., 1991b; Barbara \textit{et al}., 1993). This reflects both the small, rather heterogeneous patient populations studied and the variation in the MR localisation techniques employed. Ross and co-workers observed a significant decrease in intracerebral Pi, relative to PCr and ATP, in a group of eight cirrhotic patients with chronic hepatic encephalopathy; this they interpreted as indicating a defect in cerebral energy metabolism (Ross \textit{et al}., 1987). Barbiroli and colleagues observed a marked decrease in PCr in four patients with overt hepatic encephalopathy which was accompanied by an increase in Pi in three (Barbiroli \textit{et al}., 1992). These patients showed a significant increase in calculated ADP, a 'marked' increase in relative velocity of ATP biosynthesis and a corresponding reduction in the phosphorylation potential. Similar but less striking reductions were observed in PCr in eight cirrhotic patients who were neurologically unimpaired, three of whom also showed an increase in Pi. The same group reported 28 patients where the ratio of ATP/ADP.Pi was reduced (Barbara \textit{et al}., 1993). These changes were interpreted as evidence of abnormal brain energy metabolism. Chamuleau and co-workers observed no significant changes in cerebral $^{31}$P MR spectra acquired from 10 patients with cirrhosis and chronic hepatic encephalopathy and in particular observed no changes in intracerebral pH, PCr or ATP (Chamuleau \textit{et al}., 1991b). Similarly, in this present study, no changes were found in Pi/ATP or PCr/ATP.

The relationship between changes in cerebral energy metabolism and the pathogenesis of hepatic encephalopathy is unclear. Changes occur in cerebral energy metabolism in patients with severe hepatic encephalopathy which manifest as reductions in cerebral blood flow and
in cerebral oxygen and glucose consumption (Fazekas et al., 1956; Posner and Plum, 1960; James et al., 1969; Morgan et al., 1980). These changes reverse, following treatment, in parallel with improvements in the clinical status (Fazekas et al., 1956; Posner and Plum, 1960; Polli et al., 1969; Morgan et al., 1980). In animal models, however, changes in cerebral energy levels are delayed until after the onset of coma (Hindfelt et al., 1977) suggesting that alterations in cerebral energy metabolism may represent a secondary rather than a causal phenomenon in the genesis of the syndrome.

The findings in this study of reduced PME/ATP and PDE/ATP in the cerebral $^{31}$P MR spectra acquired from cirrhotic patients with subclinical or overt hepatic encephalopathy are, therefore, unique. The PME peak is multicomponent and includes contributions from phosphaethanolamine (PE) and phosphocholine (PC), intermediates on the pathway of phospholipid membrane synthesis (Ruiz-Cabello and Cohen, 1992), as well as contributions from AMP and glycolytic intermediates (Dagnelie et al., 1992). The PDE peak is also multicomponent and contains contributions from glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), intermediates on the pathway of phospholipid membrane breakdown (Ruiz-Cabello and Cohen, 1992), as well as a contribution from endoplasmic reticulum. These compounds cannot be separated at the magnetic field strength (1.5 Tesla) used in this study.

The reasons for the reductions that were observed in the PME/ATP and PDE/ATP ratios in the brains of patients with hepatic encephalopathy remain unclear. However, reduced contributions of choline containing compounds have been shown in the cerebral spectra of patients with hepatic encephalopathy, using proton magnetic resonance spectroscopy ($^1$H MRS) (Chamuleau et al., 1991a; Kreis et al., 1991; Kreis et al., 1992). Such changes in the choline resonance of $^1$H MR spectra may correlate with the observed reductions in PME and PDE found in the $^{31}$P MR spectra in this study, since phosphocholine is a major component
of the PME signal and glycerophosphorylcholine of the PDE signal. This suggests that cerebral phospholipid metabolism is altered in these individuals. A reduced contribution of PC to the PME peak may not be sufficient to explain the observed reductions in PME/ATP in this study, but this may also be explained, in part, by a reduced contribution of glycolytic intermediates to this peak, secondary to decreased glucose utilisation (Fazekas et al., 1956; Posner and Plum, 1960; James et al., 1969; Morgan et al., 1980; Ito et al., 1986; Mans et al., 1986; Jessy et al., 1990; Hilgier et al., 1991). Further studies using carbon-13 MRS and positron emission tomography (PET) may help clarify these findings.

Elucidation of the mechanisms responsible for the changes in cerebral $^3$P MR spectra in patients with hepatic encephalopathy may provide insights into the pathophysiology of the syndrome. The quantitative differences observed in the PME/ATP and PDE/ATP in relation to the degree of neuropsychiatric change and in response to treatment with lactitol suggests that cerebral $^3$P MRS may have a useful role, in addition to $^1$H MRS, in the diagnostic screening of cirrhotic patients and for monitoring treatment effects.
7.7 References


Table 7.1 Neuropsychiatric status of the patients undergoing $^{31}$P MR spectroscopy

Mean (range) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Age (yr)</th>
<th>Sex ratio (M:F)</th>
<th>Child C(^a) (%)</th>
<th>Mental state(^b) (0-IV)</th>
<th>Asterixis(^c) (0-IV)</th>
<th>EEG(^d) mcf (≥8.9cps)(^+)</th>
<th>Blood ammonia (μmol/ L) (11-60)(^+)</th>
<th>NCT A(^e) (15-37)(^+)</th>
<th>PSE Sum(^f) (0-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=6)</td>
<td>42.8</td>
<td>1:5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.9 (9-11)</td>
<td>27 (17-33)</td>
<td>191 (86-286)</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>(36-51)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2-4)</td>
</tr>
<tr>
<td>Subclinical (n=8)</td>
<td>50.0</td>
<td>5:3</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>9.1 (7.5-10)</td>
<td>46 (26-78)</td>
<td>135 (78-217)</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>(39-60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2-8)</td>
</tr>
<tr>
<td>Overt encephalopathy (n=19)</td>
<td>56.0</td>
<td>13:6</td>
<td>21.0</td>
<td>1-2</td>
<td>2</td>
<td>7.8 (6-10)</td>
<td>50 (24-180)</td>
<td>240 (100-286)</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>(32-67)</td>
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<td></td>
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<td></td>
<td></td>
<td>(3-16)</td>
</tr>
</tbody>
</table>

\(^{a}\)Functional grade (Pugh et al., 1973); \(^{b}\) mental state graded using West Haven criteria (Conn et al., 1977); \(^{c}\) asterixis grade (Conn et al., 1977); \(^{d}\) EEG = electroencephalogram; \(^{e}\) mcf = mean cycle frequency; \(^{f}\) NCT - Number Connection Test A Conn, 1977; \(^{g}\) PSE (Portal Systemic Encephalopathy) sum (Conn et al., 1977).

\(^{+}\)reference ranges

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Table 7.2 Comparison of metabolite ratios in the patient and reference populations

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n=15)</th>
<th>Patients (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlocalised spectra</td>
<td>Localised spectra+</td>
</tr>
<tr>
<td>TR 1000ms</td>
<td>TR 5000ms</td>
<td>TR 1000ms</td>
</tr>
<tr>
<td>PME/ATP</td>
<td>1.16 ± 0.23</td>
<td>1.29 ± 0.21</td>
</tr>
<tr>
<td>PDE/ATP</td>
<td>4.36 ± 1.17</td>
<td>4.09 ± 0.87</td>
</tr>
<tr>
<td>Pi/ATP</td>
<td>0.80 ± 0.10</td>
<td>0.90 ± 0.23</td>
</tr>
<tr>
<td>PCr/ATP</td>
<td>1.24 ± 0.45</td>
<td>1.67 ± 0.45</td>
</tr>
</tbody>
</table>

+Localised spectra were acquired from 13 healthy volunteers and 23 cirrhotic patients

Values significantly different between patient and reference populations

*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; *****p<0.0005; ******p<0.0001
Table 7.3  Comparison of metabolite ratios in the patient subgroups, graded according to neuropsychiatric status

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>PME/ATP</th>
<th>PDE/ATP</th>
<th>Pi/ATP</th>
<th>PCr/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=6)</td>
<td>1.12 ± 0.12</td>
<td>3.89 ± 0.25</td>
<td>0.80 ± 0.14</td>
<td>1.56 ± 0.33</td>
</tr>
<tr>
<td>Subclinical (n=8)</td>
<td>0.98 ± 0.16</td>
<td>3.72 ± 0.75</td>
<td>1.00 ± 0.25</td>
<td>1.60 ± 0.38</td>
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<tr>
<td>Overt CHE (n=19)</td>
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<td>3.11±0.62</td>
<td>0.83 ± 0.26</td>
<td>1.59 ± 0.41</td>
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<table>
<thead>
<tr>
<th></th>
<th>Unlocalised</th>
<th>Localised*</th>
<th>Unlocalised</th>
<th>Localised*</th>
<th>Unlocalised</th>
<th>Localised*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME/ATP</td>
<td>1.29 ± 0.12</td>
<td>1.23 ± 0.12</td>
<td>4.09 ± 0.12</td>
<td>5.19 ± 0.12</td>
<td>0.90 ± 0.12</td>
<td>0.91 ± 0.12</td>
</tr>
<tr>
<td>PDE/ATP</td>
<td>4.09 ± 0.12</td>
<td>5.19 ± 0.12</td>
<td>0.90 ± 0.12</td>
<td>0.91 ± 0.12</td>
<td>1.67 ± 0.12</td>
<td>0.95 ± 0.12</td>
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<tr>
<td>Pi/ATP</td>
<td>0.90 ± 0.12</td>
<td>0.91 ± 0.12</td>
<td>1.67 ± 0.12</td>
<td>0.95 ± 0.12</td>
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<tr>
<td>PCr/ATP</td>
<td>0.90 ± 0.12</td>
<td>0.91 ± 0.12</td>
<td>1.67 ± 0.12</td>
<td>0.95 ± 0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Localised spectra were acquired from 13 healthy volunteers and 23 patients. bMean (range) values for the reference population. Values significantly different between reference and patient populations, *p<0.05; ▲p<0.01; ▲p<0.001; ● p<0.0001
Table 7.4 Metabolite ratios for the two patients with overt encephalopathy examined before and eight weeks after the start of treatment with 20-40g of lactitol daily.

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Spectra</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>PME/ATP</td>
<td>Unlocalised (TR 5000ms)</td>
<td>1.29 (1.03-1.80) (^b)</td>
<td>0.66</td>
</tr>
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<td></td>
<td>Localised</td>
<td>1.23 (0.63-1.95) (^b)</td>
<td>0.23</td>
</tr>
<tr>
<td>PDE/ATP</td>
<td>Unlocalised (TR 5000ms)</td>
<td>4.09 (2.38-5.55) (^b)</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>Localised</td>
<td>5.19 (3.44-9.30) (^b)</td>
<td>1.82</td>
</tr>
</tbody>
</table>

\(^b\) = mean (range) values in the reference population
Figure 7.1

Unlocalised $^{31}$P MR spectrum from the entire head of a healthy adult male volunteer, acquired with TR 5000ms, 64 data collections.

PME = phosphomonoester, Pi = inorganic phosphate,
PDE = phosphodiester, PCr = phosphocreatine,
$\gamma$, $\alpha$, $\beta$ATP = adenosine triphosphate
Figure 7.2
Unlocalised spectra, (TR 5000ms) from the entire head of a healthy volunteer and a patient with overt chronic hepatic encephalopathy.
There are decreased PME/ATP and PDE/ATP ratios in the patient's spectrum.
Figure 7.3

Spectra localised to the basal ganglia (TR 1000ms) from
a) a healthy volunteer and
b) a patient with overt chronic hepatic encephalopathy.
There are decreased PME/ATP and PDE/ATP ratios in the patient's spectrum.
REGIONAL VARIATIONS IN
CEREBRAL PROTON
MAGNETIC RESONANCE SPECTROSCOPY
IN PATIENTS WITH
CHRONIC HEPATIC ENCEPHALOPATHY
8. REGIONAL VARIATIONS IN CEREBRAL PROTON MR SPECTROSCOPY IN PATIENTS WITH CHRONIC HEPATIC ENCEPHALOPATHY

8.1 Summary

Regional variations in proton magnetic resonance spectroscopy (MRS) were assessed in 26 patients and 14 healthy volunteers using a two dimensional chemical shift imaging technique. Patients were classified as being neuropsychiatically unimpaired, or as having subclinical or overt chronic hepatic encephalopathy (CHE). Peak area ratios of choline (Cho), glutamine and glutamate (Glx) and N-acetylaspartate (NAA) relative to creatine (Cr) were measured.

Significant reductions in mean Cho/Cr and elevations in mean Glx/Cr were observed in the patient population, which correlated with the severity of CHE. These results suggest that proton MRS may have diagnostic potential in this patient population.

There were also significant regional variations in these metabolite ratios with the mean Cho/Cr lowest in the occipital cortex and the mean Glx/Cr highest in the basal ganglia. NAA/Cr remained relatively constant in all areas of the brain analysed. The regional variation in the metabolite ratios suggests that spectral information from more than one voxel may be useful in the assessment of patients with CHE.

8.2 Introduction

Magnetic resonance spectroscopy (MRS) allows the study of cerebral metabolism in vivo. Phosphorus-31 MRS provides information on cerebral phospholipids, sugar phosphates, phosphocreatine and ATP (Coutts et al., 1989), while proton MRS can be utilised to provide
information on brain metabolites such as choline, creatine, N-acetyl aspartate, myoinositol, glutamine and glutamate (Ross et al., 1992).

A number of proton MRS studies of the brain have been undertaken in cirrhotic patients with chronic hepatic encephalopathy (CHE). These show a reduction in the choline (Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) and myoinositol resonances (Kreis et al., 1990; Bruhn et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) and an increase in the composite glutamine/glutamate resonances (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) obtained from single voxels containing cerebral cortex.

However, information from the basal ganglia, in addition to the cerebral cortex, is also of interest since a proportion of patients with CHE display extrapyramidal abnormalities. Furthermore, MR imaging studies have shown areas of high signal from this region of the brain in these patients (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992).

In this study the use of a two dimensional chemical shift imaging (2-D CSI) technique is described. This allowed the mapping of different areas of the brain and the assessment of regional variations in proton MR spectra.

8.3 Aims

1. To assess regional variations in proton MR spectra of the brain in patients with biopsy-proven cirrhosis.

2. To relate any changes observed to the patients' neuropsychiatric status.
8.4 Methods

Thirty patients with biopsy-proven cirrhosis of varying functional severity and a reference population of 14 healthy volunteers were evaluated for proton MRS study. Spectroscopic information on four patients was unavailable owing to subject movement, claustrophobia or an inability to lie flat for the full examination time. The patient population therefore comprised 26 individuals, 14 men and 12 women, of mean (range) age 49 (21-70) years. Seventeen patients had alcohol related liver disease, four biliary cirrhosis, three postviral cirrhosis, one autoimmune chronic active hepatitis/cirrhosis and one cryptogenic cirrhosis. Functionally, 11 patients were Child's grade A, eight were Child's grade B and seven were Child's grade C (Pugh et al., 1973). All patients had been abstinent from alcohol for a minimum of 3 months and none was receiving psychoactive medication.

The reference population comprised eight healthy men and six healthy women of mean (range) age 49.4 (31-71) years. None drank alcohol in excess of 20 g/day and none was taking regular medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant. Ethical approval was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC 93/4047) and the Royal Free Hospital and School of Medicine, London. All subjects provided written informed consent.

8.4.1 Patient Assessment

All patients were clinically stable at the time of the MRS study. A full neurological, psychometric and electrophysiological assessment was performed on all patients within 24 hours of the MRS examination. The mental state of each patient was assessed using West
Haven criteria (Conn et al., 1977). A battery of four psychometric tests were employed, under standardised conditions, comprising Number Connection Tests (NCT) A and B (Conn, 1977), the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). The same observer was used throughout the study. Electroencephalograms (EEG) were performed using conventionally placed electrodes and a mean cycle frequency was obtained. Immediately prior to the MRS examination, blood was drawn for standard biochemical parameters of liver function. Blood ammonia concentrations were also measured using a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co. Ltd. Kyoto, Japan) (Quero et al., 1993). These data were used to calculate a PSE sum (Conn et al., 1977), which reflected the severity of the encephalopathy.

The patient population was classified into three groups on the basis of the psychometric analysis, EEG mean cycle frequency and the clinical assessment (Table 8.1). Five individuals were classified as being neuropsychiatrically unimpaired; they had no history of CHE and showed no neuropsychiatric abnormalities when assessed. Ten individuals were classified as having subclinical hepatic encephalopathy; they had no history of CHE and were clinically normal on neurological examination, but showed slowing of their EEG mean cycle frequency and/or impaired performance in at least two of the four psychometric tests employed. One patient in this group was on maintenance treatment with the non-absorbable disaccharide, lactitol (Morgan et al., 1989). The remaining 11 patients either had untreated overt CHE (n=2) or else gave a history of overt CHE requiring long-term maintenance treatment with a non-absorbable disaccharide: lactulose or lactitol (n=9). Five of these patients displayed a mild rest tremor, but there were no other neurological abnormalities.
8.4.2 MR Methods

Cerebral proton MR spectra were obtained using a Picker prototype spectroscopy system (Picker International, Cleveland, Ohio), based on a whole body magnet (Oxford Magnet Technology, Oxford, UK), operating at 1.5 Tesla. An enveloping quadrature transmit/receive coil tuned to 64MHz, was employed for all examinations. 

T₁-weighted axial images were acquired in order to position a 2cm transverse slice at the level of the basal ganglia. A 2-D CSI technique was used to obtain spectra from multiple contiguous voxels covering all the brain in the selected slice. CSI spectral acquisition consisted of a 1331 - 180° spin echo; a 1331 composite pulse for water suppression, with a 90° excitation at the N-acetylaspartate (NAA) resonance, (intra-pulse spacing 2.1ms), a slice selective 180° and phase encoding in the two in-plane directions. The sequence acquisition parameters were TR 1500ms, TE 130ms and 32 phase-encoding steps in each direction giving 1024 averages in 26 minutes. The CSI resolution was 20mm x 10mm x 20mm to give a nominal voxel size of 4cc. In addition, a non-selective inversion pulse preceded each data acquisition, TI 150ms, to reduce the fat signal from the surface voxels and consequent bleeding into neighbouring voxels. Shimming was performed on the water signal from the slice, typically achieving a line-width at half-height of 5 Hz. The total examination time was approximately 90 minutes.

8.4.3 Data Processing

The 2-D CSI spectra were processed with an exponential filter of 120ms (2.7 Hz) in the time domain and cosine filtering in each spatial direction. Data were zero filled to 2048 points in the time domain, prior to Fourier transformation. All spectra were manually phased.

Previous studies (Kreis et al., 1990; Bruhn et al, 1991; Chamuleau et al., 1991; Kreis et al.,
1991; Kreis et al., 1992, Ross et al., 1992) have focused on single voxels which contained variable amounts of cerebral cortex.

Information from the basal ganglia was also of interest, since imaging studies have shown areas of high signal from this region of the brain (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992). Therefore, spectra localised to the basal ganglia, the occipital cortex and temporal cortex were analysed in every case; each voxel was selected by an observer, blinded to the clinical status of all subjects (S.D. T.-R.).

A knowledge-based algorithm (Saeed, 1993) was used both for removal of the water peak residuum and for baseline flattening. As cerebral creatine has been reported not to vary significantly in patients with CHE, using MR methods (Kreis et al., 1991), this resonance was chosen as an internal reference standard. Peak area ratios of NAA, Cho and Glx relative to Cr were measured by another blinded observer, using the NMR1® spectral processing program (New Methods Research, Inc, E. Syracuse, N.Y.) on a SUN SPARCstation 10 (Sun Microsystems, Inc, Mountain View, C.A.).

8.4.4 Statistical Analysis

Since the data were not normally distributed, non-parametric tests were used for all statistical analyses. Overall regional variations in metabolite ratios were assessed using a Friedman analysis of variance, while comparisons between individual regions of the brain were made using Wilcoxon rank correlations with a Bonferroni correction. Values for the metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test. Comparisons between the patient subgroups were assessed using the Kruskal-Wallis test, while comparisons between the individual patient subgroups and the
reference population were made using the Mann Whitney U test with a Bonferroni correction where necessary. In all cases a p value of <0.05 was considered significant.

8.5 Results

A typical cerebral proton MR spectrum (Figure 8.1) contains resonances which can be assigned to the methyl moieties of choline (Cho) at 3.22 ppm, creatine (Cr) at 3.02 ppm and N-acetyl aspartate (NAA) at 2.02 ppm. The chemical shift of these resonances varied by less than 0.04 ppm in the areas of the brain analysed.

The region of the spectrum between 2.1 and 2.5 ppm includes contributions from both glutamine and glutamate. These resonances have strong homonuclear coupling and are complex multiplet structures which are not easily separated at 1.5T. This composite resonance is referred to as Glx (Kreis et al., 1991). In aqueous solution, the component Glx resonances are largely refocussed at an echo time of 130 ms and have the appearance of a single, broad Gaussian-type peak with the sequences used (Bryant et al., 1994, Taylor-Robinson et al., 1994). Such appearances are the same as those seen in the spectra in vivo (Figure 8.1) and therefore for the work described in this chapter, the resolved spectral lines (NAA, Cr, Cho and Glx) were quantified with Gaussian curve-fitting. Other bell-shaped functions, such as Lorentzian, were also investigated, but Gaussian fitting gave the best results in terms of fit and reproducible ratios. A myoinositol resonance is not evident, because this region of the spectrum is only minimally excited with the sequences used in this study.

There were significant regional variations in the Cho/Cr and Glx/Cr metabolite ratios in each subject (p<0.05). The NAA/Cr showed no significant regional variation in any individual (Table 8.2).
In healthy volunteers the mean Cho/Cr was lowest in the occipital cortex and the mean Glx/Cr lowest in the basal ganglia. The NAA/Cr was relatively constant in all the voxels examined (Table 8.2).

In the patient population the mean Cho/Cr was also lowest in the occipital cortex (Figure 8.2) and NAA/Cr was relatively constant in all three areas of the brain (Table 8.2). However, in contrast to the findings in healthy individuals, the mean Glx/Cr was highest in the basal ganglia (Table 8.2).

Overall, the mean Glx/Cr was significantly higher and the mean Cho/Cr significantly lower in the patient population than in the reference population in all three areas of the brain studied (Table 8.2). There were no significant differences in NAA/Cr between patients and healthy volunteers (Table 8.2).

The reduction in Cho/Cr and elevation in mean Glx/Cr observed in the patient population reflected the degree of neuropsychiatric impairment (Figure 8.3, Table 8.3). Patients who were classified as neuropsychiatrically unimpaired had essentially normal spectra (Figure 8.3). Patients with overt CHE had a significantly lower mean Cho/Cr than healthy individuals, in all areas of the brain studied (Table 8.3).

The mean Glx/Cr in these patients was significantly higher than in both the healthy volunteers and the neuropsychiatrically unimpaired patients (Table 8.3). The elevation in Glx/Cr was greatest in the basal ganglia (p<0.005) (Table 8.3). Patients with subclinical hepatic encephalopathy had less pronounced abnormalities in Cho/Cr and Glx/Cr than patients with overt CHE, but these changes were not significantly different from the other two patient groups (Table 8.3).

There were no significant correlations between the patients' spectral abnormalities and any of the individual parameters measured: liver function tests, Child's grading, psychometric performance, EEG mean cycle frequency, blood ammonia concentrations or the PSE sum.
8.6 Discussion

In the present study, significant regional variations in cerebral Glx/Cr and Cho/Cr metabolite ratios were observed in proton MR spectra in all subjects studied. A multi-voxel technique was employed to study regional variations. The binomial 1331 solvent suppression method we used is known to be tolerant of radiofrequency (rf) amplitude (Hore, 1983). However, analysis of the spectra in the regions of interest have demonstrated that resonances do not vary significantly in relationship to this rf variation. The observed proton resonances are partially saturated to a variety of extents and this does not vary significantly between voxels. In addition resonances such as myoinositol, which are close to the unwanted water resonances, can be severely attenuated; the extent depending upon their $T_1$ and $T_2$.

Sequences were employed in this study which give a 90° excitation at the NAA resonance, ~200 Hz from water at 1.5T, hence an absence of myoinositol and a higher NAA/Cr than sequences involving CHESS suppression techniques (Kreis et al., 1991; Kreis et al., 1992).

In healthy volunteers the mean Cho/Cr was lowest in the occipital cortex and the mean Glx/Cr lowest in the basal ganglia. The NAA/Cr remained relatively constant throughout the brain. Variation in cerebral metabolite concentrations have been observed in healthy individuals previously (Frahm et al., 1989; Kreis et al., 1993). The results differ depending on the percentage distribution of white and grey matter in the voxels chosen.

Thus, Frahm and colleagues (Frahm et al., 1989) studied voxels in the insular area containing mainly grey matter, the occipital area containing mainly white matter, the thalamus and the cerebellum. They assumed a cerebral total creatine concentration of 10mM and calculated the concentration of choline in 14 healthy volunteers to be lowest in the insular area. The thalamus was observed to have the highest concentration of choline. Kreis and co-workers (Kreis et al., 1993) looked at voxels containing mostly white matter from the
parietal cortex and mostly grey matter from the occipital cortex in 22 healthy subjects. They observed a lower concentration of choline in the occipital voxels.

Details of regional variations in glutamine/glutamate were not provided in either study, but variations in NAA concentrations were observed in the voxels selected in both studies. Frahm and colleagues (Frahm et al., 1989) found NAA to be lowest in thalamus and cerebellum. Kreis and co-workers (Kreis et al., 1993) calculated the NAA and creatine concentrations to be highest in voxels from the occipital cortex and lowest in voxels from the parietal cortex.

The results of the present study are not directly comparable to other previous studies, because the areas of the brain studied, the size of voxels selected and the MR sequences used were different.

In the total patient population of the present study, there was a significant reduction in mean Cho/Cr and a significant elevation in mean Glx/Cr in all regions of the brain when compared to the healthy volunteers. The magnitude of these changes differed significantly in each part of the brain analysed. There were no significant regional variations in NAA/Cr in the total patient group and no significant differences in the magnitude of this ratio from healthy volunteers.

In the patient population, the regional variations in Cho/Cr in the three brain areas studied mirrored the pattern observed in the reference population, while the regional variation in Glx/Cr was significantly altered. The highest values for this metabolite ratio were observed in the basal ganglia, which was the area where the lowest Glx/Cr values were observed in healthy individuals. Overall the patients showed significantly increased Glx/Cr and significantly reduced Cho/Cr in all brain areas studied. These changes reflected the patients' neuropsychiatric status, to a degree, but there was considerable overlap between patient
subgroups. Thus, Glx/Cr values clearly distinguished the patients with overt CHE from those who were neuropsychiatrically unimpaired, but not from those with subclinical impairment. However, it is noteworthy that patients with no neuropsychiatric impairment had essentially normal spectra. It should also be noted that the majority of patients with overt CHE in the present study were on long term maintenance treatment for their encephalopathy and that this may have attenuated the cerebral findings. The distinction between the groups may have been more pronounced if these patients had been studied in their untreated state, but such patients are rarely encountered at a time when they would be sufficiently stable to allow MRS examinations. Furthermore, it would be unethical to stop treatment in patients once stable.

At 1.5T, the glutamine MR resonance is difficult to separate from the glutamate resonance. In the published studies (Kreis et al., 1990; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992), there is agreement that the composite glutamine/glutamate peak is elevated in patients with CHE.

Abnormalities in cerebral glutamine/glutamate and ammonia metabolism are considered to be of pathogenic importance in CHE (Butterworth et al., 1987). Glutamate is the major excitatory neurotransmitter in the brain and it is reduced in patients with CHE (Butterworth et al., 1987). Glutamine, an amino acid formed by amidation of glutamate, plays an important role in ammonia detoxification in the brain, but may itself be neurotoxic (Butterworth et al., 1987). Cerebral glutamine levels are considerably elevated in hyperammonaemic states such as CHE (Butterworth et al., 1987). In animals with encephalopathy, inhibition of cerebral glutamine synthetase and hence reduction in cerebral glutamine concentrations, results in an improvement in the condition (Hawkins et al., 1993). Similarly in man, treatment of hepatic encephalopathy is aimed at reducing circulating
ammonia concentrations and hence by extrapolation cerebral glutamine concentrations (Ferenci, 1991). Ammonia cannot be detected by proton spectroscopy because of rapid proton exchange with water. However, changes observed in the glutamine/glutamate region of the spectrum in chronic CHE may reflect ammonia incorporation into glutamine. Blood ammonia levels are not always elevated in these patients (Stahl, 1963) and do not necessarily correlate with symptoms (Fischer, 1992). The condition is heterogeneous and changes in other implicated toxins such as the mercaptans (Zieve et al., 1974) and in different neurotransmitters, such as serotonin (Hawkins et al., 1987), dopamine (Morgan et al., 1980) or norepinephrine (Fischer, 1992) which are not assessable by MRS methods, may be of greater or lesser importance in individual patients.

There is little data on regional variations in Glx in CHE because single voxel techniques have been used in previous studies (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992). However, Bruhn and colleagues (Bruhn et al., 1991) found that in two-thirds of 22 patients studied, Glx was generally higher in grey matter than white matter.

In the current study, there were regional variations of mean Glx/Cr in the patient population which were most significantly different from the reference population in the basal ganglia (deep grey matter). On $T_1$-weighted MR imaging increased signal is observed in the basal ganglia of such patients (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992).

The reasons behind this hyperintensity are not fully understood, but may represent an accumulation of Alzheimer type II astrocytes (Norenberg, 1987), which are the principal site of glutamate conversion to glutamine in hyperammonaemia (Butterworth et al., 1987). The regional differences observed in Glx/Cr may therefore be dependent on astrocyte function.
A reduction in cerebral choline has been observed in all published MRS series of CHE patients (Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992), but regional variations in choline have not been documented previously. Ross and colleagues (Kreis et al., 1991) made the point that in healthy volunteers, Cho/Cr varies considerably between grey and white matter, making this metabolite ratio susceptible to any changes in the grey/white matter distribution in any single voxel examined. Such changes in voxel composition could contribute to the regional variations of Cho/Cr we noted in the patient and reference population and to some of the discrepancies we observed between individuals in each of our patient subgroups. Despite a scatter in results, the reduction in mean Cho/Cr in the patient population in our study appeared to be correlated with neuropsychiatric status.

The relative contribution of choline containing compounds to the choline MR peak is incompletely understood, although cell membrane precursors such as phosphocholine are major components of this resonance (Miller, 1991). A reduction in the cerebral choline resonance in CHE may reflect changes in cerebral phospholipid metabolism (Miller, 1991). The function of blood brain barrier transport mechanisms may be altered in CHE (James et al., 1978), but animal studies showed that the physical integrity of this barrier remains (Hawkins et al., 1987) despite any changes in cerebral phospholipids.

Ross and colleagues (Kreis et al., 1990; Kreis et al., 1991; Kreis et al., 1992; Ross et al., 1992) reported an abnormal myoinositol resonance in patients with this condition, in spectra acquired with a single voxel STEAM sequence; TR 1500/TE 30ms, voxel size 12-27cm³ (Kreis et al., 1991; Kreis et al., 1992). The significance of such findings remains unclear. No changes in the myoinositol resonance were able to be reported in the current study because of the nature of the MR sequence used (vide supra), but regional variations in other metabolites were observed in all patients.
These changes were not consistent in every patient and this suggests data from a single area of the brain should be treated with caution and that information from a number of areas of the brain should be considered in individual cases.

8.7 Conclusions

There are two conclusions which may be drawn from this study:

1. The changes in proton MR spectra which correlate with the severity of the underlying hepatic encephalopathy may have diagnostic potential, particularly in the difficult area of subclinical disease, where patient monitoring and gauging response to treatment modalities is not always straightforward.

2. The results imply that a multivoxel MRS technique may be useful in the assessment of patients with chronic hepatic encephalopathy. The magnitude of the changes in Glx/Cr in the basal ganglia suggests that this area of the brain should be included in routine examinations.
8.8 References


Table 8.1  Neuropsychiatric status of the patients undergoing proton MRS

Mean (range) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Age (yr)</th>
<th>Sex ratio (M:F)</th>
<th>Mental state (0-IV)</th>
<th>Asterixis (0-4)</th>
<th>EEG mcf (≥8.9 cps)</th>
<th>NCT A (15-37)</th>
<th>Blood ammonia μmol/l (11-60)</th>
<th>PSE sum (0-28)</th>
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<tbody>
<tr>
<td>Unimpaired (n=5)</td>
<td>51.6</td>
<td>2:3</td>
<td>0</td>
<td>0</td>
<td>10.1 (9-12)</td>
<td>31 (26-35)</td>
<td>154 (105-238)</td>
<td>3.0 (2-4)</td>
</tr>
<tr>
<td>Subclinical (n=10)</td>
<td>45.1</td>
<td>5:5</td>
<td>0</td>
<td>0</td>
<td>8.5 (6-9)</td>
<td>42 (24-65)</td>
<td>207 (86-286)</td>
<td>4.6 (1-9)</td>
</tr>
<tr>
<td>Overt CHE (n=11)</td>
<td>51.3</td>
<td>7:4</td>
<td>1-2</td>
<td>2 (0-3)</td>
<td>7.4 (6-9)</td>
<td>69 (28-121)</td>
<td>181 (99-286)</td>
<td>12.1 (6-16)</td>
</tr>
</tbody>
</table>

*Mental state graded using West Haven criteria (Conn et al., 1977); *asterixis grade (Conn et al., 1977); *EEG = electroencephalogram; mcf = mean cycle frequency; *NCT - Number Connection Test A (Conn, 1977); *PSE (Portal Systemic Encephalopathy) sum (Conn et al., 1977).
+reference ranges
Table 8.2  Comparison of metabolite ratios in the patient and reference populations

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n=14)</th>
<th>Total patient population (n=26)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
<td>Occipital cortex</td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>2.07 ± 0.50</td>
<td>2.11 ± 0.45</td>
<td>2.17 ± 0.35</td>
<td>2.55 ± 0.92</td>
<td>2.14 ± 0.62</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.88 ± 0.25</td>
<td>0.89 ± 0.28</td>
<td>0.72 ± 0.26*</td>
<td>0.61 ± 0.30**</td>
<td>0.56 ± 0.23**</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>0.09 ± 0.03b</td>
<td>0.13 ± 0.13</td>
<td>0.15 ± 0.12</td>
<td>0.53 ± 0.67***** d</td>
<td>0.33 ± 0.31*</td>
</tr>
</tbody>
</table>

Reference population:
Values significantly different between occipital cortex and other regions, * p<0.05.
Values significantly different between the basal ganglia and other regions, b p<0.05.

Patient population:
Values significantly different between the occipital cortex and other regions, c p<0.05.
Values significantly different between the basal ganglia and the other regions, d p<0.05.
Values significantly different between patient and reference populations:
*p<0.05; **p<0.01; ***p<0.005; ****p<0.001; *****p<0.0005.
Table 8.3 Comparison of regional metabolite ratios in the patient subgroups, graded according to neuropsychiatric status

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>NAA/Cr</th>
<th>Cho/Cr</th>
<th>Glx/Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
<td>Occipital cortex</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
<td>Occipital cortex</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
<td>Occipital cortex</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>2.07 ± 0.50*</td>
<td>2.11 ± 0.45*</td>
<td>2.17 ± 0.35*</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>0.88 ± 0.25*</td>
<td>0.89 ± 0.28*</td>
<td>0.72 ± 0.26*</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>0.09 ± 0.03*</td>
<td>0.13 ± 0.13*</td>
<td>0.15 ± 0.12*</td>
</tr>
<tr>
<td>Unimpaired (n=5)</td>
<td>1.97 ± 0.37</td>
<td>2.19 ± 0.68</td>
<td>2.52 ± 0.34</td>
</tr>
<tr>
<td>Subclinical (n=10)</td>
<td>2.27 ± 0.37</td>
<td>2.08 ± 0.48</td>
<td>2.37 ± 0.79</td>
</tr>
<tr>
<td>Overt CHE (n=11)</td>
<td>2.53 ± 0.83</td>
<td>2.18 ± 0.75</td>
<td>2.29 ± 0.63</td>
</tr>
</tbody>
</table>

* reference range

Values significantly different between reference and patient populations, *p<0.05; ** p<0.01; ● p<0.005.
Values significantly different between neuropsychically unimpaired patients and the other patient groups, ■ p<0.05.
Figure 8.1

2-D CSI spectrum, (TR 1500ms, TE 130ms) from the basal ganglia of a healthy volunteer. Cho = choline (3.22ppm), Cr = creatine (3.02ppm), NAA = N-acetylaspartate (2.02ppm), Glx = glutamine/glutamate (2.1-2.5ppm).
Figure 8.2

2-D CSI spectra (TR 1500ms, TE 130ms) from
a) the basal ganglia
b) the temporal cortex
c) the occipital cortex of a patient with overt chronic hepatic encephalopathy.
There is a marked reduction in the Cho/Cr ratio in the occipital cortex.
Basal ganglia

Temporal cortex

Occipital cortex

3.0 2.0 ppm 1.0

Naa

Cr

Glx

Cho
Figure 8.3

2-D CSI spectra (TR 1500ms, TE 130ms) from the basal ganglia of
a) a normal volunteer
b) a neuropsychiatrically unimpaired patient
c) a patient with subclinical chronic hepatic encephalopathy
d) a patient with overt chronic hepatic encephalopathy.

There is an increase in the Glx/Cr ratio and a decrease in the Cho/Cr ratio with increasing neuropsychiatric impairment.
MR IMAGING OF THE BASAL GANGLIA IN CHRONIC LIVER DISEASE:
CORRELATION OF $T_1$-WEIGHTED AND MAGNETISATION TRANSFER CONTRAST MEASUREMENTS WITH LIVER DYSFUNCTION AND NEUROPSYCHIATRIC STATUS
9. MR IMAGING OF THE BASAL GANGLIA IN CHRONIC LIVER DISEASE: CORRELATION OF T$_1$-WEIGHTED AND MAGNETISATION TRANSFER CONTRAST MEASUREMENTS WITH LIVER DYSFUNCTION AND NEUROPSYCHIATRIC STATUS

9.1 Summary

The purpose of this study was to correlate the changes in the basal ganglia seen on T$_1$-weighted magnetic resonance imaging (MRI) of the brain in chronic liver disease with indices of liver dysfunction and neuropsychiatric status. Conventional T$_1$-weighted spin echo (T$_1$WSE) and T$_1$-weighted magnetization transfer (MT) images were obtained in 26 patients with biopsy-proven cirrhosis (nine Child's grade A, 10 Child's grade B and seven Child's grade C). Four subjects showed no evidence of neuropsychiatric impairment on clinical, psychometric and electrophysiological testing, seven showed evidence of subclinical hepatic encephalopathy and 15 were classified as having overt hepatic encephalopathy. Signal intensities of basal ganglia nuclei (head of caudate, putamen, globus pallidus and thalamus) and adjacent brain parenchyma were measured and contrast calculated. On T$_1$WSE imaging, contrast measurements of the globus pallidus were significantly greater in patients with neuropsychiatric dysfunction than in those who were unimpaired (p<0.05). This was not observed in the other basal ganglia nuclei. Patients with subclinical and overt hepatic encephalopathy could not be distinguished on the basis of contrast measurements of the globus pallidus or of any other nucleus. T$_1$WSE contrast measurements of the globus pallidus were increased with elevations in blood ammonia levels (p<0.05) and with the severity of liver dysfunction, when graded according to the Pugh's score (p<0.05). Those patients with the worst liver injury (Child's grade C) had significantly greater T$_1$WSE pallidal contrast measurements (p<0.05) than those patients with minimal liver injury (Child's grade A). The patients with intermediate liver damage (Child's grade B) could not be distinguished from the other two groups. While MT imaging highlighted the basal ganglia and showed a correlation between globus pallidus contrast and blood ammonia levels (p<0.05), no other relationship between MT contrast measurements and either the degree of hepatic encephalopathy or the severity of liver dysfunction was found.
9.2 Introduction

Previous magnetic resonance imaging (MRI) studies of the brain in patients with chronic liver disease have demonstrated high signal intensity in the basal ganglia, particularly in the globus pallidus (Brunberg et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994). These observations have been correlated with indicators either of liver dysfunction or chronic hepatic encephalopathy (CHE). However, there is no consensus of opinion as to the precise clinical or biochemical factors which correlate with the MRI appearances. Differences may have arisen between studies because of heterogeneity of patient populations, differences in classification of neuropsychiatric status and variations in the timing of neuropsychiatric assessments with respect to the MRI examination.

9.3 Aims

The aims of this study were to measure the changes observed on MRI of the basal ganglia in chronic liver disease and to correlate the quantified signal intensities with the clinical indices of the patients' neuropsychiatric status and the functional severity of their liver disease. Some of the previous studies have not quantified change in signal intensity. Contrast measurements were therefore used as a continuous variable in order to assess the relationship between signal intensity in the basal ganglia and both the neuropsychiatric status and indices of hepatic impairment. In order to increase the conspicuity of the deep grey matter, T1W magnetization transfer (MT) images (Hajnal et al., 1992) were used in addition to standard T1W spin echo techniques.
9.4 Patients and Methods

The study population comprised 26 individuals (15 men and 11 women) of mean age 52 (range 21-70) years with biopsy-proven cirrhosis. Thirteen patients had alcohol related liver disease, eight biliary cirrhosis, two cryptogenic cirrhosis, two postviral cirrhosis, and one autoimmune chronic active hepatitis/cirrhosis. Twenty-five patients had previously been documented as having oesophageal varices. Twelve individuals had a history of variceal bleeding and six of these had subsequently undergone surgical portacaval shunting. All patients had been abstinent from alcohol for a minimum of 3 months. None of the patients had a history of head injury or cerebrovascular disease and none was receiving psychoactive medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant. Ethical approval was obtained from the Ethics Committee of the Royal Postgraduate Medical School, London (REC 93/3995). All individuals studied provided written informed consent.

9.4.1 Patient Assessment

A full neurological, psychometric and electrophysiological assessment was performed in all patients on the same day as the MRI study. The mental state of each patient was assessed using West Haven criteria (Conn et al., 1977). Psychometric performance was assessed by the same observer under standardised conditions, using a battery of four tests comprising Number Connection Tests (NCT) A and B (Conn, 1997), the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). Electroencephalograms (EEG) were performed using conventionally placed electrodes and a mean cycle frequency obtained. Blood ammonia concentrations were measured using a Blood Ammonia Checker
II (Kyoto Daichi Kagaku Co, Ltd, Kyoto, Japan) (Quero et al., 1993). These data were used to calculate a PSE (portal systemic encephalopathy) sum (Conn et al., 1977), which reflected the severity of the encephalopathy.

The patients were classified into three groups on the basis of the psychometric analysis, EEG mean cycle frequency and the clinical assessment (Table 9.1). Four individuals were classified as being neuropsychiatrically unimpaired; they had no history of CHE and showed no neuropsychiatric abnormalities when assessed. Seven individuals were classified as having subclinical hepatic encephalopathy. They had no history of CHE and were clinically normal on examination, but showed slowing of their EEG mean cycle frequency to below the reference range for an alert adult of ≥8.9 cycles per second (cps) and/or impaired performance (beyond our reference range from normal healthy volunteers) in at least two of the four psychometric tests employed. One patient in this group was on maintenance treatment with the non-absorbable disaccharide, lactitol (Morgan et al., 1989). The remaining 15 patients either had overt untreated CHE (n=1) or else gave a history of overt CHE, which was episodic or persistent, requiring long-term maintenance treatment with the non-absorbable disaccharides, lactulose (n=4) or lactitol (n=10) (Morgan et al., 1989). The mean length of time since the diagnosis of encephalopathy was 43 months (range 3-266 months). Thirteen of these patients displayed a mild rest tremor, but there were no other neurological abnormalities.

Blood was drawn for standard biochemical and haematological parameters of liver function and a Pugh's score (Pugh et al., 1973) and Child's grade (as modified by Pugh) (Pugh et al., 1973), reflecting the severity of hepatic dysfunction were calculated for each subject. Subjects were then classified according to the severity of liver dysfunction (Table 9.2). Functionally, nine (34.6%) were Child's grade A, 10 (38.5%) Child's grade B and seven (26.9%) Child's grade C.
9.4.2 Imaging Methods

MRI of the basal ganglia was performed on a 1.0 T Picker Vista HPQ system (Picker International, Cleveland, Ohio, USA). Conventional transverse $T_1$-weighted spin echo ($T_1$WSE) images were obtained using repetition times (TR) 580-760 ms, echo time (TE) 20 ms with a slice thickness of 6 mm, phase resolution 128 x 256 and two excitations. $T_1$-weighted magnetization transfer (MT) images were also obtained with the same sequence parameters, by the addition of a saturating radiofrequency irradiation pulse with a frequency offset of 1000 Hz, amplitude of 11.5 $\mu$T and a 56% duty cycle (Hajnal et al., 1992).

9.4.3 Image Analysis

The basal ganglia nuclei (the head of the caudate nucleus, the putamen, the globus pallidus and the thalamus) were visually assessed by one observer who was blinded to the patient's clinical condition, the conspicuity of each nucleus being graded as normal or increased by comparison to the appearance of images from normal volunteers. Signal intensities (SI) of the same nuclei and the adjacent white matter (WM) were measured using scanner resident software. The nucleus to background contrast was then calculated using the formula:

$$\frac{SI_{\text{nucleus}} - SI_{\text{WM}}}{SI_{\text{nucleus}} + SI_{\text{WM}}}$$

No signal intensity measurements were made in six of the patients with overt encephalopathy because their MR images were grossly degraded by motion artefact.
9.4.4 Statistical Analysis

Non-parametric tests were used for all statistical analyses, because the data were not normally distributed. Comparisons of both $T_1$WSE and MT contrast measurements between the patient subgroups, ranked according to the presence/severity of encephalopathy or the severity of liver dysfunction (Child's grade), were assessed using a non-parametric analysis of variance (Kruskal-Wallis). Comparisons of both $T_1$WSE and MT contrast measurements between individual patient subgroups were made using the Mann Whitney U test with a Bonferroni correction where appropriate. Spearman Rank correlations were calculated to determine the relationship between the continuous neuropsychiatric and hepatic variables, including the Pugh's score, and the quantitative measures of basal ganglia signal. In all cases a $p$ value of $<0.05$ was considered significant.

9.5 Results

On visual assessment of the conventional $T_1$WSE images, there was bilateral, symmetrical hyperintensity of the globus pallidus (Figure 1) in 17 patients (65%) and of the putamen in five patients (19%), compared to normal volunteers. There was a significant difference ($p<0.05$) in the $T_1$WSE contrast measurements of the globus pallidus between the non-encephalopathic individuals and those with subclinical and overt CHE, when the latter two groups were considered both separately and together (Table 9.3). This difference was not seen in any of the other basal ganglia measured (Table 9.3). In patients with subclinical encephalopathy, the $T_1$WSE contrast measurements of all the nuclei, including the globus pallidus, were not significantly different from those of patients with overt CHE (Table 9.3).
There was no relationship between the T₁WSE contrast measurements and the length of time since individual patients were diagnosed with encephalopathy. Similarly, there was no association between the contrast measurements in the basal ganglia and the EEG mean cycle frequency, psychometric performance or the PSE sum.

There was a significant relationship between the measured contrast in the globus pallidus on T₁WSE imaging and blood ammonia levels (p<0.05). There was also a significant association between the T₁WSE contrast measurements of the globus pallidus and the severity of liver dysfunction, when patients were classified according to Pugh's score (p<0.05). In those patients with minimal liver injury (Child's grade A), the contrast measurements in the globus pallidus were significantly less (p<0.05) than in those patients with the worst liver function (Child's grade C). Patients with intermediate liver damage (Child's grade B) could not be distinguished from the other two groups.

There was no significant relationship between the measured pallidal contrast on T₁WSE imaging and the prothrombin time, serum albumin or standard biochemical liver function tests. Furthermore, there was no correlation between the T₁WSE contrast measurements of the other basal ganglia nuclei and any index of liver dysfunction.

There was also no relationship between contrast measurements of any of the nuclei including the globus pallidus on conventional T₁WSE imaging and the presence of asterixis or rest tremor, the presence of portasystemic shunting and/or oesophageal varices or a history of previous variceal bleeding.

T₁-weighted magnetization transfer (MT) images showed the basal ganglia nuclei with greater conspicuity in normal volunteers than on standard T₁WSE imaging (Figure 9.2). On visual assessment of our patient group, 14 of the 17 patients noted to have pallidal hyperintensity on conventional T₁WSE imaging, also showed striking bilateral, symmetrical pallidal hyperintensity on MT imaging (Figure 9.2, Table 9.4). This
exaggeration of the normal pattern was also seen in the putamen in four patients and in
the head of the caudate nucleus in two patients. A significant relationship was noted
between blood ammonia levels and the MT contrast measurements in the globus pallidus
(p<0.05). This was not observed in any of the other nuclei. However, MT contrast
measurements failed to discriminate between patient groupings when subjects were
classified according to the presence or severity of encephalopathy using the West Haven
criteria (Table 9.4), or the degree of liver dysfunction using the Child-Pugh score.

9.6 Discussion
The globus pallidus appeared bilaterally and symmetrically hyperintense on conventional
T1WSE images and showed a significant increase in measured contrast with the presence,
but not the severity, of encephalopathy. Pallidal T1WSE contrast measurements were also
significantly related to the severity of liver dysfunction. None of these relationships could
be demonstrated for any of the other basal ganglia.

On visual assessment, MT imaging highlighted all the basal ganglia nuclei, but none of
the MT contrast measurements discriminated between patients graded according to the
severity of liver dysfunction or the neuropsychiatric status. However, pallidal MT
contrast measurements were significantly associated with elevations in blood ammonia
levels.

Pallidal hyperintensity has been noted by previous investigators (Brunberg et al., 1991;
Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky
et al., 1992; Pujol et al., 1993; Norton et al., 1994) on T1-weighted imaging in 56-100% of
patients with chronic liver disease. In the majority of these studies (Brunberg et al.,
1991; Inoue et al., 1991; Pujol et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993;
Norton et al., 1994) no significant relationship was observed between the increased signal
intensity and the patients' neuropsychiatric status at the time of MRI examination. However, the clinical, electrophysiological and neuropsychiatric assessments in this study were performed on the same day as the MRI examination, whereas this was not achieved in other studies. Zeneroli and colleagues (Zeneroli et al., 1991) noted that the majority of patients with encephalopathy had these imaging changes, but no correlation was made between the magnitude of the MRI abnormalities and the degree of neuropsychiatric impairment. In the present study, while there was a significant difference in pallidal contrast measurements between neuropsychiatically normal and encephalopathic patients on T1WSE imaging, no difference could be found between the groups with subclinical and overt encephalopathy.

Pujol and colleagues (Pujol et al., 1991; Pujol et al., 1993) found no correlation between the globus pallidus hyperintensity and the patients' current mental state or EEG findings, but observed a significant correlation with previous episodes of hepatic encephalopathy (Pujol et al., 1993). There was also a significant correlation with the presence of rest tremor. Kulisevsky and colleagues (Kulisevsky et al., 1992) found a relationship between the imaging appearances in the globus pallidus and a history of recurrent hepatic encephalopathy. Dysdiadochokinesis, the presence of primitive reflexes, postural tremor, EEG slowing and performance in certain psychometric tests were also related to pallidal changes by these authors. These results differ from the present study, in that no association between T1WSE contrast measurements and EEG mean cycle frequency, psychometric performance, the PSE sum or the presence of rest tremor was observed. This may be because the patient populations and methods of patient assessment were different.

In the present study group, on T1WSE imaging, the measured pallidal contrast correlated significantly with the severity of liver dysfunction, as indicated by the Child-Pugh score.
There was also a significant relationship with elevations in blood ammonia levels, but not with the presence of portasystemic shunting and/or oesophageal varices or a history of previous variceal bleeding. None of these relationships was observed in the other basal ganglia nuclei.

In contrast, there was no significant relationship between the presence of globus pallidus hyperintensity and either the aetiology or functional severity of liver disease reported in most studies (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992), but Pujol and colleagues (Pujol et al., 1993) did find a significant correlation between these MRI findings and the Child-Pugh score. They concluded that MRI abnormalities in these patients are associated with the severity of liver failure, but unlike the present study, these authors also found a correlation with previous episodes of variceal bleeding as well as with abnormalities in various biochemical liver function tests.

Kulisevsky and co-workers (Kulisevsky et al., 1992) noted a correlation between the globus pallidus signal and blood ammonia levels. They also observed that the signal was higher in patients with portasystemic shunts. Similarly, Inoue and colleagues observed in their study (Inoue et al., 1991) that the nine subjects who showed globus pallidus hyperintensity all had large portal-systemic collateral vessels, receiving blood from the superior mesenteric vein. However, no signal intensity measurements were made in this series.

The cause of the increased signal intensity in the basal ganglia of CHE patients on $T_1$-WSE imaging is uncertain. Reversal of pallidal hyperintensity has been described in patients following liver transplantation (Pujol et al., 1991; Pujol et al., 1993), indicating that these changes are closely linked to hepatic function. However, signal increases in the basal ganglia have also been observed on $T_1$-weighted images in patients with systemic
lupus erythematosus (Brunberg et al., 1991), neurofibromatosis (Mirowitz et al., 1989), and those receiving long-term parenteral nutrition (Mirowitz et al., 1991), indicating that these abnormalities are characteristic, but not specific to patients with chronic hepatic failure. Furthermore, Gupta and colleagues (Gupta et al., 1993) reported on three patients with acute hepatic failure, one of whom also had pallidal hyperintensity.

Gut derived toxins, such as ammonia are implicated in the pathogenesis of CHE (Cooper et al., 1987; Schenker and Brady 1994). Elevations in blood ammonia may be consequent to hepatic failure (Kulisevsky et al., 1992) or to portasystemic shunting (Ferenci, 1991). It is interesting to note the correlation between blood ammonia levels and the MRI signal of the globus pallidus on T₁-weighted imaging, observed by Kulisevsky and colleagues (Kulisevsky et al., 1992) and on both T₁WSE and MT imaging in the current study.

Proton magnetic resonance spectroscopy (MRS) has been used to demonstrate regional variations in cerebral metabolites in patients with chronic hepatic encephalopathy (see chapter 8). Peak area ratios of glutamine and glutamate relative to creatine were most elevated in the basal ganglia. Ammonia cannot be detected by proton MRS because of rapid proton exchange with water. However, changes in the glutamine/glutamate region of the spectrum reflect ammonia incorporation into glutamine. The changes in these metabolites, most obvious in the basal ganglia, may be consequent to regional differences in astrocyte function. The changes observed in the basal ganglia on T₁-weighted imaging in patients with CHE may parallel the functional abnormalities observed with proton MRS.

In animal models of hepatic encephalopathy, an abnormal accumulation of type II Alzheimer cells has been demonstrated in the basal ganglia (Norenberg, 1987). These astrocytes play an important role in ammonia detoxification in the brain (Butterworth et al., 1987) and may be responsible for the MRI changes in the globus pallidus of patients with chronic liver failure (Inoue et al., 1991). These abnormal cells have large, pale
nuclei, prominent nucleoli and are rich in mitochondria, rough endoplasmic reticulum and cytoplasmic vacuolation (Voorhies et al., 1983). This proliferation of cellular organelles in response to hyperammonaemia may alter signal intensity on T₁-weighted images by increasing intracellular membrane content. A limited number of post-mortem studies has been undertaken on patients dying with hepatic failure, who showed cerebral MRI abnormalities during life. Kulisevsky and co-workers (Kulisevsky et al., 1992) reported two patients in whom Alzheimer type II cells were noted in the caudate, putamen and the globus pallidus. In CHE, there may be regional differences in astrocyte function in response to circulating toxins (Norenberg, 1981). However, these astrocytes are not confined to the basal ganglia (Norenberg, 1981) and it would therefore seem unlikely that the presence of these cells are solely responsible for the T₁ shortening on T₁WSE images.

Pallidal demyelination (Kulisevsky et al., 1992) and lipid droplets (McConnell and Castaldo 1990; Kulisevsky et al., 1992) have also been noted in post-mortem studies. Lipid accumulation has been suggested as the cause of the signal abnormalities in the globus pallidus (Zeneroli et al., 1991) and circulating short chain fatty acids have been implicated in the pathogenesis of hepatic encephalopathy (Chen et al., 1970), but post-mortem studies have only demonstrated small quantities of lipid in the basal ganglia of these patients (Victor et al., 1965).

Oxidative damage resulting from free-radical production has been suggested as the central mechanism in a number of neurodegenerative diseases of the basal ganglia including Huntington's disease and progressive supranuclear palsy (Jenner, 1994). Such oxidative stress may well occur in CHE, resulting in lipid peroxidation and the observed changes on T₁-weighted imaging. However, the free-radical species involved and the cause of increased free-radical production, have yet to be identified.

The deposition of materials such as melanin, manganese and deoxyhaemoglobin can
cause $T_1$ shortening and this may be responsible for hyperintensity on $T_1$-weighted images. The results of most post-mortem studies in patients dying of chronic liver failure have not shown increased deposition of these substances in the basal ganglia (Victor, 1965; Levy et al., 1984; Kulisevsky et al., 1992). However, Pomier-Layrargues and colleagues (Pomier-Layrargues et al., 1995) found increased manganese concentrations in the globus pallidus of nine patients who died in hepatic coma. They suggested that these abnormalities in the basal ganglia may be responsible for the extrapyramidal symptoms seen in patients with hepatic encephalopathy and the pallidal hyperintensity seen on MRI.

Signal changes other than in the globus pallidus have been reported in CHE. Inoue observed increased signal in portions of the internal capsule (Inoue et al., 1991), while Brunberg noted similar changes in the mesencephalon surrounding the red nucleus, quadrigeminal plate and anterior pituitary (Brunberg et al., 1991). In neither of these studies was the contrast of the basal ganglia quantified in the various clinical groups. Norton and colleagues (Norton et al., 1994) quantified signal differences between patients with CHE and normal controls in both the limbic and the extrapyramidal systems and also the associated myelinated pathways. However, no correlation was made between signal intensity and the severity of encephalopathy or liver dysfunction. On visual assessment of $T_1$WSE images, hyperintensity was observed in the putamen of five patients, while on similar assessment of MT images both the putamen and the head of the caudate nucleus appeared brighter than normal in a small minority of individuals.

MT imaging has not previously been reported in patients with CHE. This technique highlighted the basal ganglia and improved lesion conspicuity, but MT contrast measurements did not add to the information obtained from $T_1$WSE images. Furthermore, because in normal subjects, the basal ganglia are hyperintense on MT images, the further increase in signal associated with CHE may be less easily recognised than corresponding
changes on T₁-weighted images.

It is obvious that there has been no consensus of opinion as to the precise factors which correlate with the hyperintensity seen in the basal ganglia of patients with chronic liver disease, but the current study is the first to correlate T₁,WSE imaging contrast measurements with both the severity of liver dysfunction and the presence, although not the severity, of encephalopathy. T₁,WSE imaging may act as an indicator of exposure to circulating toxins in CHE, where the pathogenesis is multifactorial and its relationship to the severity of liver disease is complex. Further studies are required to elucidate the cause of the changes in the basal ganglia seen on T₁-weighted imaging. These may include a combination of MRI and MRS to evaluate the functional changes behind these observations.
9.7 References


Table 9.1 Neuropsychiatric classification of patients undergoing MRI examination

Mean (range) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Age (yr)</th>
<th>Sex ratio (M:F)</th>
<th>Mental state (0-IV)</th>
<th>Asterixis (0-IV)</th>
<th>EEG (^c) mcf (≥8.9cps) (^d)</th>
<th>NCT A (^d) (15-37) (^d)</th>
<th>Blood ammonia (μmol/l) (11-60)</th>
<th>PSE(^e) Sum (0-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=4)</td>
<td>46.5 (40-52)</td>
<td>1:3</td>
<td>0</td>
<td>0</td>
<td>9.5 (9-10)</td>
<td>30 (26-34)</td>
<td>151 (91-190)</td>
<td>3.0 (1-4)</td>
</tr>
<tr>
<td>Subclinical (n=7)</td>
<td>45.8 (21-64)</td>
<td>4:3</td>
<td>0</td>
<td>0</td>
<td>8.9 (7.5-10)</td>
<td>48 (37-65)</td>
<td>211 (86-286)</td>
<td>4.4 (1-6)</td>
</tr>
<tr>
<td>Overt CHE (n=15)</td>
<td>56.2 (32-70)</td>
<td>10:5</td>
<td>1-2</td>
<td>2 (1-3)</td>
<td>7.1 (4.5-9.5)</td>
<td>67 (23-145)</td>
<td>200 (98-286)</td>
<td>12.0 (6-22)</td>
</tr>
</tbody>
</table>

\( ^{a}\)Mental state graded using West Haven criteria (Conn et al., 1977); \(^{b}\)asterixis grade (Conn et al., 1977); \(^{c}\)EEG = electroencephalogram; mcf = mean cycle frequency; \(^{d}\)NCT - Number Connection Test A (Conn, 1977); \(^{e}\)PSE (Portal Systemic Encephalopathy) sum (Conn et al., 1977).

Table 9.2 Relationship of neuropsychiatric status to severity of liver dysfunction

198
### Table 9.2  Relationship of neuropsychiatric status to severity of liver dysfunction

Mean (range) values (+reference ranges)

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>Pugh's score (^a)</th>
<th>Child's grade (^a)</th>
<th>serum bilirubin (\mu \text{mol/L}) ((5-17)^+)</th>
<th>plasma albumin (\text{g/L}) ((35-50)^+)</th>
<th>prothrombin time (\text{s}) ((12-14)^+)</th>
<th>serum alkaline phosphatase (\text{u/L}) ((35-130)^+)</th>
<th>serum aspartate transaminase (\text{u/L}) ((5-40)^+)</th>
<th>serum (\gamma) glutamyl-transpeptidase (\text{u/L}) ((10-48)^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired ((n=4))</td>
<td>7 ((5-9))</td>
<td>A-B</td>
<td>86 ((10-249))</td>
<td>40 ((28-52))</td>
<td>14 ((14))</td>
<td>225 ((59-471))</td>
<td>64 ((22-103))</td>
<td>208 ((36-364))</td>
</tr>
<tr>
<td>Subclinical encephalopathy ((n=7))</td>
<td>7 ((5-11))</td>
<td>A-C</td>
<td>136 ((18-544))</td>
<td>34 ((26-44))</td>
<td>14.7 ((13-17))</td>
<td>284 ((48-793))</td>
<td>115 ((36-199))</td>
<td>141 ((26-449))</td>
</tr>
<tr>
<td>Overt encephalopathy ((n=15))</td>
<td>8 ((5-12))</td>
<td>A-C</td>
<td>90 ((8-616))</td>
<td>37 ((26-52))</td>
<td>17.5 ((12-34))</td>
<td>223 ((69-1553))</td>
<td>76 ((29-236))</td>
<td>167 ((16-1006))</td>
</tr>
</tbody>
</table>

* Degree of functional hepatic impairment (Pugh et al., 1973)

+ reference ranges

199
Table 9.3  $T_1$-weighted contrast measurements in each of the basal ganglia nuclei

mean (range) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>Globus pallidus</th>
<th>Head of caudate</th>
<th>Putamen</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=4)</td>
<td>+0.001 (-0.018 to +0.019)</td>
<td>-0.027 (-0.07 to +0.009)</td>
<td>-0.023 (-0.04 to -0.006)</td>
<td>-0.037 (-0.07 to -0.02)</td>
</tr>
<tr>
<td>Subclinical encephalopathy (n=7)</td>
<td>+0.059* (-0.012 to +0.11)</td>
<td>-0.018 (-0.063 to +0.022)</td>
<td>-0.002 (-0.043 to +0.025)</td>
<td>-0.06 (-0.11 to -0.03)</td>
</tr>
<tr>
<td>Overt encephalopathy (n=9)</td>
<td>+0.096* (-0.016 to +0.53)</td>
<td>-0.024 (-0.073 to +0.041)</td>
<td>-0.008 (-0.063 to +0.047)</td>
<td>-0.06 (-0.095 to -0.012)</td>
</tr>
</tbody>
</table>

significant difference between neuropsychiatrically unimpaired patients and the other two groups: * p<0.05
Table 9.4 Magnetization transfer contrast measurements in each of the basal ganglia nuclei

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Globus pallidus</th>
<th>Head of caudate</th>
<th>Putamen</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=4)</td>
<td>+0.067 (+0.013 to +0.11)</td>
<td>+0.065 (+0.031 to +0.089)</td>
<td>+0.063 (+0.029 to +0.081)</td>
<td>-0.05 (-0.056 to +0.032)</td>
</tr>
<tr>
<td>Subclinical encephalopathy</td>
<td>+0.131 (+0.044 to +0.19)</td>
<td>+0.049 (+0.003 to +0.1)</td>
<td>+0.065 (+0.024 to +0.1)</td>
<td>-0.073 (-0.16 to -0.015)</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overt encephalopathy</td>
<td>+0.112 (+0.02 to +0.17)</td>
<td>+0.05 (-0.001 to +0.077)</td>
<td>+0.49 (-0.038 to +0.074)</td>
<td>-0.055 (-0.13 to +0.03)</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 9.1 a & b

$T_1$-weighted spin echo images of the basal ganglia (TR 580-760 ms, TE 20 ms) from:
a) a healthy volunteer
b) a patient with overt hepatic encephalopathy showing bilateral symmetrical hyperintensity of the globus pallidus.
Figure 9.2 a & b

$T_1$-weighted magnetization transfer images of the basal ganglia (TR 580-760ms, TE 20 ms; frequency offset 1000Hz, amplitude 11.5μT, 56% duty cycle) from:
a) a normal volunteer,
b) a patient with overt hepatic encephalopathy.
All the basal ganglia are highlighted with most striking changes in the globus pallidus.
MR IMAGING AND SPECTROSCOPY
OF THE BASAL GANGLIA IN CHRONIC
LIVER DISEASE:
CORRELATION OF $T_1$-WEIGHTED
CONTRAST MEASUREMENTS
WITH ABNORMALITIES IN PROTON AND
PHOSPHORUS-31 MR SPECTRA
10. **MR IMAGING AND SPECTROSCOPY OF THE BASAL GANGLIA IN CHRONIC LIVER DISEASE: CORRELATION OF T<sub>1</sub>-WEIGHTED CONTRAST MEASUREMENTS WITH ABNORMALITIES IN PROTON AND PHOSPHORUS-31 MR SPECTRA**

10.1 **Summary**

The purpose of this study was to correlate the hyperintensity in the globus pallidus seen on T<sub>1</sub>-weighted magnetic resonance imaging (MRI) of the brain in chronic liver disease with changes in metabolite ratios measured from both proton and phosphorus-31 magnetic resonance spectroscopy (MRS) localised to the basal ganglia. T<sub>1</sub>-weighted spin echo (T<sub>1</sub>WSE) images were obtained in 21 patients with biopsy-proven cirrhosis (nine Child's grade A, eight Child's grade B and four Child's grade C). Four subjects showed no evidence of neuropsychiatric impairment on clinical, psychometric and electrophysiological testing, four showed evidence of subclinical hepatic encephalopathy and 13 had overt hepatic encephalopathy. Signal intensities of the globus pallidus and adjacent brain parenchyma were measured and contrast calculated, which correlated with the severity of the underlying liver disease, when graded according to the Pugh's score (p<0.05). Proton MRS of the basal ganglia was performed in 12 patients and 14 healthy volunteers. Peak area ratios of choline (Cho), glutamine and glutamate (Glx) and N-acetylaspartate relative to creatine (Cr) were measured. Significant reductions in mean Cho/Cr and elevations in mean Glx/Cr ratios were observed in the patient population. Phosphorus-31 MRS (31P MRS) of the basal ganglia was performed in the remaining nine patients and in 15 healthy volunteers. Peak area ratios of phosphomonoesters (PME), inorganic phosphate, phosphodiesters (PDE) and phosphocreatine relative to βATP (ATP) were then measured. Mean values of PME/ATP and PDE/ATP were significantly lower in the patient population. No correlation was found...
between the $T_1$-WSE MRI contrast measurements of the globus pallidus and the abnormalities in the metabolite ratios measured from either proton or phosphorus-31 MR spectra. The results of this study suggest that pallidal hyperintensity seen on $T_1$-WSE MR imaging of patients with chronic liver disease is not related to the functional abnormalities of the brain observed in hepatic encephalopathy.

10.2 Introduction

Magnetic resonance imaging (MRI) studies of the brain in patients with chronic liver disease have demonstrated hyperintensity in the basal ganglia as a frequent finding in 37%-100% of subjects examined with $T_1$-weighted sequences (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994; Krieger et al., 1995; Thuluvath et al., 1995). The underlying cause of these abnormalities remains a point of conjecture, although in the study outlined in the previous chapter, it was found that the increase in signal intensity from the globus pallidus correlates with the severity of liver dysfunction, but not the severity of chronic hepatic encephalopathy (CHE).

Magnetic resonance spectroscopy (MRS) allows the study of cerebral metabolism in vivo. Proton MRS can be utilised to provide information on brain metabolites such as choline, creatine, N-acetyl aspartate, myoinositol, glutamine and glutamate (Ross et al., 1992), while $^{31}$P MRS provides information on cerebral phospholipids, sugar phosphates, and high energy phosphates such as phosphocreatine and ATP (Coutts et al., 1989).

A number of proton MRS studies of the brain have been undertaken in patients with CHE. These show a reduction in the choline (Chamuleau et al., 1991a; Kreis et al., 1991; Kreis et al., 1992; Ross et al., 1992; Ross et al., 1994) and myoinositol resonances (Kreis et al., 1992).
1990; Bruhn et al., 1991; Kreis et al., 1991; Kreis et al., 1992; Ross et al., 1992; Haussinger et al., 1994; Ross et al., 1994) and an increase in the composite glutamine/glutamate resonances (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991a; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992; Ross et al., 1994) in voxels containing cerebral cortex. In chapter 8, similar abnormalities in voxels localised to the basal ganglia were also demonstrated, which correlate with the severity of the neuropsychiatric impairment.

Similarly, a number of $^{31}$P MR studies of the brain have been undertaken in patients with CHE (Ross et al., 1987; Luyten et al., 1989; Chamuleau et al., 1991b; Barbiroli et al., 1992; Barbara et al., 1993; Taylor-Robinson et al., 1994a), but there has been no real consensus of opinion as to the spectral abnormalities found in this patient group. In chapter 7, it was found that ratios of phosphomonoester (PME) and phosphodiester (PDE) to ATP were significantly reduced in spectra localised to the basal ganglia. These findings correlated with the patients' neuropsychiatric status. The most marked reductions in these metabolite ratios were observed in patients with overt CHE.

10.3 Aims

The aims of this study were to measure the hyperintensity of the globus pallidus on T$_1$-weighted spin echo (T$_1$ WSE) MRI of the basal ganglia in chronic liver disease and to correlate the quantified signal intensities with the MRS abnormalities measured in both proton and $^{31}$P MR spectra localised to the same region of the brain.

10.4 Methods

Twenty-five patients with biopsy-proven cirrhosis of varying functional severity were evaluated for MRI and MRS study. Spectroscopic information on four patients was
unavailable owing to subject movement, claustrophobia or an inability to lie flat for the full examination time. The patient population therefore comprised 21 individuals, 11 men and 10 women, of mean (range) age 49.5 (21-70) years. Nine patients had alcohol related liver disease, six biliary cirrhosis, two postviral cirrhosis, two autoimmune chronic active hepatitis/cirrhosis and two cryptogenic cirrhosis. All patients had been abstinent from alcohol for a minimum of 3 months and none was receiving psychoactive medication.

The reference population for proton MRS comprised 14 healthy volunteers (eight men and six women) of mean (range) age 49.4 (31-71) years, while the reference population for $^{31}$P MRS comprised 15 healthy volunteers (seven men and eight women) of mean (range) age 40.8 (21-67) years. None drank alcohol in excess of 20 g/day and none was taking regular medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant. Ethical approval was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC 93/4047 and REC 93/3995) and the Royal Free Hospital and School of Medicine, London. All subjects provided written informed consent.

10.4.1 Patient Assessment

All patients were clinically stable at the time of the MR study. A full neurological, psychometric and electrophysiological assessment was performed on all patients within 24 hours of the MRI and MRS examinations. The mental state of each patient was assessed using West Haven criteria (Conn et al., 1977). Psychometric performance was assessed by the same observer under standardised conditions, using a battery of four tests comprising Number Connection Tests (NCT) A and B (Conn, 1977), the Digit Symbol subtest of the
Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). Electroencephalograms (EEG) were performed using conventionally placed electrodes and the mean cycle frequency was obtained. Blood ammonia concentrations were measured using a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co, Ltd, Kyoto, Japan) (Quero et al., 1993). These data were used to calculate a PSE (portal systemic encephalopathy) sum (Conn et al., 1977), which reflected the severity of the encephalopathy.

The patients were classified into three groups on the basis of the psychometric analysis, EEG mean cycle frequency and the clinical assessment (Table 10.1). Four individuals were classified as being neuropsychiatrically unimpaired; they had no history of CHE and showed no neuropsychiatric abnormalities when assessed. Four individuals were classified as having subclinical hepatic encephalopathy. They had no history of CHE and were clinically normal on examination, but showed slowing of their EEG mean cycle frequency to below the reference range for an alert adult of ≥8.9 cycles per second (cps) and/or impaired performance (beyond our reference range from normal healthy volunteers) in at least two of the four psychometric tests employed. One patient in this group was on maintenance treatment with the non-absorbable disaccharide, lactitol (Morgan et al., 1989). The remaining 13 patients either had overt untreated CHE (n=1) or else gave a history of overt CHE, which was episodic or persistent, requiring long-term maintenance treatment with the non-absorbable disaccharides, lactulose (n=4) or lactitol (n=10) (Morgan et al., 1989). Twelve of these patients displayed a mild rest tremor, but there were no other neurological abnormalities.
Blood was drawn for standard biochemical and haematological parameters of liver function and a Pugh's score (Pugh et al., 1973) and Child's grade (as modified by Pugh) (Pugh et al., 1973), reflecting the severity of hepatic dysfunction were calculated for each subject. Subjects were then classified according to the severity of liver dysfunction (Table 10.2). Functionally, nine (43%) were Child's grade A, eight (38%) Child's grade B and four (19%) Child's grade C.

10.4.2 MR Imaging Methods

MRI of the basal ganglia was performed on a 1.0 T Picker Vista HPQ system (Picker International, Cleveland, Ohio). Transverse T1WSE images were obtained using repetition times (TR) 580-760 ms, echo time (TE) 20 ms with a slice thickness of 6 mm, phase resolution 128 x 256 and two excitations.

10.4.3 Image Analysis

The globus pallidus was visually assessed by one observer who was blinded to the patient's clinical condition, the conspicuity of each nucleus being graded as normal or increased by comparison to the appearance of images from normal volunteers. Signal intensities (SI) of the same nuclei and the adjacent white matter (WM) were measured using scanner resident software. The nucleus to background contrast was then calculated using the formula:

\[
\frac{SI_{\text{nucleus}} - SI_{\text{WM}}}{SI_{\text{nucleus}} + SI_{\text{WM}}}
\]
10.4.4 MRS Methods

Proton and $^{31}$P MR spectra of the brain were obtained using a Picker prototype spectroscopy system, based on a whole body magnet (Oxford Magnet Technology, Oxford, UK), operating at 1.5 Tesla.

10.4.4.1 Proton MRS examinations

An enveloping quadrature transmit/receive coil tuned to 64MHz, was employed for all proton MRS examinations. $T_1$-weighted axial images were acquired in order to position a 2cm transverse slice at the level of the basal ganglia. A two-dimensional chemical shift imaging (2-D CSI) technique was used to obtain spectra from multiple contiguous voxels covering all the brain in the selected slice. CSI spectral acquisition consisted of a 1331 - 180° spin echo; a 1331 composite pulse for water suppression, with a 90° excitation at the N-acetylaspartate (NAA) resonance, (intra-pulse spacing 2.1ms) a slice selective 180° and phase encoding in the two in-plane directions. The sequence acquisition parameters were TR 1500ms, TE 130ms and 32 phase-encoding steps in each direction giving 1024 averages in 26 minutes. The CSI resolution was 20mm x 10mm x 20mm to give a nominal voxel size of 4cc. In addition, a non-selective inversion pulse preceded each data acquisition, TI 150ms, to reduce the fat signal from the surface voxels and consequent bleeding into neighbouring voxels. Shimming was performed on the water signal from the slice, typically achieving a line-width at half-height of 5 Hz. The total proton MRS examination time was approximately 90 minutes.
10.4.4.2 Phosphorus-31 MRS examinations

An enveloping saddle-shaped transmitter coil and a separate saddle-shaped receiver coil were employed for all $^{31}$P MRS examinations. Both these coils were double-tuned for phosphorus and proton frequencies at 25.9 MHz and 64 MHz respectively; the proton signal being used for shimming and to acquire $T_1$-weighted axial imaging to verify spectral localisation.

Spectra localised to the basal ganglia were acquired, using a 3-D CSI technique (Coutts et al., 1989). A total of 512 voxels in a cubic array, $8 \times 8 \times 8$ in dimensions, covering the whole head was acquired, with each voxel containing a conventional MR absorption spectrum. A total of 2048 averages were acquired for the 3-D CSI examination at a TR of 1000 ms; a nominal spatial resolution of $(3 \text{cm})^3$ was obtained in approximately 34 minutes.

A 45° radio-frequency (rf) pulse was employed, which was calibrated using an external pick-up loop. This enabled the rf field to be monitored directly. The variations in loading upon the transmitter system between phantom calibration studies and in vivo human studies were corrected for by this procedure. The choice of a 45° rf pulse is commensurate with improving spectral appearances or signal-to-noise ratio (SNR) in instances where TR is sufficiently low that resonances are partially saturated (Ernst and Anderson, 1966). The full $^{31}$P MRS protocol for this study, as described above, took approximately 90 minutes.

10.4.5 MRS Data Processing

The proton MR spectra were processed with an exponential filter of 120 ms (2.7 Hz) in the time domain and cosine filtering in each spatial direction. Data were zero filled to 2048 points in the time domain, prior to Fourier transformation. All spectra were manually phased. A knowledge-based algorithm (Saeed, 1993) was used both for removal of the water peak.
residuum and for baseline flattening. As cerebral creatine has been reported not to vary significantly in patients with CHE, using MR methods (Kreis et al., 1991), this resonance was chosen as an internal reference standard. Peak area ratios of NAA, choline (Cho) and glutamine/glutamate (Glx) relative to creatine (Cr) were measured using the NMR1® spectral processing program (New Methods Research, Inc, E. Syracuse, N.Y.) on a SUN SPARCstation 10 (Sun Microsystems, Inc, Mountain View, C.A.). The components of the Glx resonance have strong homonuclear coupling and are complex multiplet structures, which are not easily separated at 1.5T. In aqueous solution, these resonances are largely refocussed at an echo time of 130 ms and have the appearance of a single, broad Gaussian-type peak with the sequences used in this study (Bryant et al., 1994). For the work described in this chapter, the resolved spectral lines (NAA, Cr, Cho and Glx) have been quantified with Gaussian curve-fitting. Other bell-shaped functions, such as Lorentzian, were also investigated, but Gaussian fitting gave the best results in terms of fit and reproducible metabolite ratios.

The $^{31}$P MR spectra were processed with a cosine filter in all three spatial directions, a 30 ms exponential filter and were manually phased. Spatial localisation ensures that fast decaying signals (broad resonances) are absent from the resultant localised spectra. The baseline roll, resulting from this delay in data acquisition while phase-encoding gradients were applied in the 3-D CSI sequences, was removed using a knowledge-based algorithm (Saeed and Menon; 1993). A manual baseline correction was used, where necessary, and peak areas ratios of phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE) and phosphocreatine (PCr) relative to βATP were measured using the NMR1® spectral processing program. The data were fitted to inverse polynomial functions.
10.4.6 Statistical Analysis

The 95% confidence intervals for the individual metabolite ratios in the healthy volunteers were used to define the reference range for both proton and $^{31}$P MRS. Values outside this range were considered abnormal. Values for the metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test, since the data were not normally distributed. Correlations between $T_1$WSE contrast measurements of the globus pallidus and indices of underlying liver disease or abnormalities in MRS metabolite ratios from the basal ganglia were made using Spearman rank correlations. In all cases a p value of <0.05 was considered significant.

10.5 Results

10.5.1 MR Imaging

On visual assessment of the $T_1$WSE images, there was bilateral, symmetrical hyperintensity of the globus pallidus (Figure 1) in 12 patients (57%), when compared to normal volunteers.

There was a significant correlation between the $T_1$WSE contrast measurements of the globus pallidus and the severity of liver dysfunction, when patients were classified according to Pugh's score ($p<0.05$). In patients with minimal liver injury (Child's grade A), the contrast measurements in the globus pallidus were significantly less than in patients with intermediate liver damage (Child's grade B) ($p<0.05$) or than in those with the worst liver function (Child's grade C) ($p<0.05$). The latter two groups could not be distinguished from each other.

There was no significant relationship between the measured pallidal contrast on $T_1$WSE imaging and the prothrombin time, serum albumin or standard biochemical liver function tests. There was no significant difference in the $T_1$WSE contrast measurements of the globus...
pallidus between the non-encephalopathic individuals and those with subclinical and overt CHE (Table 10.3).

There was no association between the contrast measurements in the globus pallidus and the EEG mean cycle frequency, psychometric performance, blood ammonia levels or the PSE sum.

10.5.2 MR Spectroscopy

A typical proton MR spectrum of the brain (Figure 10.2) contains resonances which can be assigned to the methyl moieties of choline (Cho) at 3.22 ppm, creatine (Cr) at 3.02 ppm and NAA at 2.02 ppm. The region of the spectrum between 2.1 and 2.5 ppm, labelled Glx, includes contributions from both glutamine and glutamate (Kreis et al., 1991). A myoinositol resonance is not evident, because this region of the spectrum is only minimally excited with the sequences used in this study.

Overall, in spectra localised to the basal ganglia, the mean Glx/Cr ratio was significantly higher (p<0.005) and the mean Cho/Cr ratio was significantly lower (p<0.005) in the patient population than in the reference population (Figure 10.3, Table 10.4). There were no significant differences in NAA/Cr ratios between patients and healthy volunteers (Table 10.4).

There were no significant correlations between the proton MR spectral abnormalities in the patient population and any of the individual parameters measured: liver function tests, Child's grading, psychometric performance, EEG mean cycle frequency, blood ammonia concentrations or the PSE sum.

A typical $^{31}$P MR spectrum from the basal ganglia of a healthy volunteer contains at least seven resonances (Bottomley et al., 1984), which can be assigned to phosphomonoesters.
(PME), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr), ATP, ATP and βATP (Figure 10.4). The PME and PDE peaks are multicomponent, the ATP peak contains contributions from αADP and NADH and the ATP contains contributions from βADP.

In the patient population, significant reductions were observed in the mean PME/ATP ratio (p<0.01) and the mean PDE/ATP ratio (p<0.01) in spectra localised to the basal ganglia (Figure 10.5, Table 10.5). There were no consistent changes in the mean PCr/ATP or mean Pi/ATP ratios in the patient population and therefore no significant difference from the reference population (Table 10.5).

There were no significant correlations between any of the $^{31}$P MR spectral metabolite ratios and the patients' liver function tests, Child's grading, EEG mean cycle frequency, psychometric performance, blood ammonia concentrations or the PSE sum.

10.5.3 MR Imaging and Spectroscopy

In the patient population, no correlation was found between the $T_1$WSE MRI contrast measurements of the globus pallidus and the reduction in the Cho/Cr or elevation in Glx/Cr ratios measured in the proton MR spectra localised to the basal ganglia. Similarly, there was no correlation between the reductions in PME/ATP and PDE/ATP ratios measured from phosphorus-31 MR spectra localised to the basal ganglia and the $T_1$WSE MRI contrast measurements of the globus pallidus.

10.6 Discussion

On $T_1$WSE imaging, the globus pallidus appeared bilaterally and symmetrically hyperintense in 57% of the patients with chronic liver disease in this study. Pallidal $T_1$WSE contrast
measurements were significantly related to the severity of liver dysfunction, as measured by the Child-Pugh scoring system.

When compared to healthy volunteers, the patient population in this study exhibited a significant reduction in the mean Cho/Cr ratio and a significant elevation in the mean Glx/Cr ratio measured from proton MR spectra localised to the basal ganglia, while in the $^{31}$P MR spectra, significant reductions in the PME/ATP and PDE/ATP ratios were measured.

No correlation was found between the $T_1$WSE MRI contrast measurements of the globus pallidus and the abnormalities in the metabolite ratios measured from either proton or $^{31}$P MR spectra.

Previous MRI studies of the brain in patients with chronic liver disease have demonstrated hyperintensity of the globus pallidus in 37%-100% of subjects examined with $T_1$-weighted sequences (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994; Thuluvath et al., 1995), but there has been no real consensus of opinion as to the clinical or biochemical factors which correlate with these MRI findings in this patient group. Differences may have arisen between studies because of the small, but heterogeneous study populations, and because of differences in MR methodology and clinical classification.

The underlying cause of this increased MRI signal intensity in the basal ganglia in these patients also remains uncertain. Pujol and colleagues described reversal of pallidal hyperintensity in patients following liver transplantation (Pujol et al., 1991; Pujol et al., 1993), suggesting that these changes are closely associated with hepatic function. In addition, basal ganglia hyperintensity has been observed on $T_1$-weighted images in patients with other conditions such as neurofibromatosis (Mirowitz et al., 1989), systemic lupus erythematosus (Brunberg et al., 1991), occupational manganese toxicity (Nelson et al., 1993) and in patients
receiving long-term parenteral nutrition (Mirowitz et al., 1991). These abnormalities are therefore characteristic of, but not specific to, patients with chronic hepatic failure. Furthermore, increased signal from the globus pallidus has also been reported in one patient with acute hepatic failure (Gupta et al., 1993).

In both the current study and in the previous chapter (chapter 9), the measured pallidal contrast correlated significantly with the severity of liver dysfunction, as indicated by the Child-Pugh score. Pujol and colleagues (Pujol et al., 1993) also reported a significant correlation between these MRI abnormalities in patients with chronic liver disease and the Child-Pugh score. In contrast, no significant relationship was reported between the presence of globus pallidus hyperintensity and either the aetiology or functional severity of liver disease in most studies (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992, Thuluvath et al., 1995).

The deposition of materials such as melanin, manganese and deoxyhaemoglobin can cause T1 shortening and therefore may be responsible for hyperintensity on T1-weighted images, but the results of most post-mortem studies in patients dying of chronic liver failure have not shown increased deposition of these substances in the basal ganglia (Victor, 1965; Levy et al., 1984; Kulisevsky et al., 1992). More recent studies have focussed on manganese deposition in the basal ganglia in chronic liver disease. Pomier-Layrargues and colleagues (Pomier-Layrargues et al., 1995) found increased manganese concentrations in the globus pallidus of nine patients who died in hepatic coma. They suggested that these abnormalities in the basal ganglia may be responsible for the extrapyramidal symptoms seen in patients with hepatic encephalopathy and the pallidal hyperintensity seen on MRI. Krieger and colleagues (Krieger et al., 1995) correlated these MRI changes with elevations in whole blood manganese levels in 10 patients with Child's grade C cirrhosis. Three of these patients
subsequently died and at post-mortem there were increased tissue concentrations of manganese in the basal ganglia. The highest manganese concentrations were in the caudate nucleus, followed by the quadrigeminal plate and then the globus pallidus. Occupational manganese exposure may cause manganese encephalopathy (Nelson et al., 1993) and affected individuals exhibit extrapyramidal symptoms which mimic Parkinson’s disease and are similar to the symptoms displayed by certain patients with CHE. Although the metabolism of manganese is not fully understood, patients with chronic liver disease may have impaired biliary manganese excretion and therefore elevated total body manganese levels (Versieck et al., 1974). Krieger and colleagues (Krieger et al., 1995) suggested that the increased basal ganglia manganese levels in these patients may be responsible for the neurotoxic features of hepatic encephalopathy. However, manganese deposition is not confined to the globus pallidus and it would seem unlikely that this is the sole factor responsible for the MRI appearances, which are mainly pallidal.

In the previous MRI study (chapter 9), pallidal contrast measurements were correlated with the presence, but not the severity of CHE. There were significant differences between neuropsychiatically normal and encephalopathic patients on T,WSE imaging, but no difference could be found between patients with subclinical and overt CHE. However, in the current study no distinction could be made on the basis of neuropsychiatric status. Similarly, no significant relationship was observed between the increased signal intensity and the patients' neuropsychiatric status at the time of MRI examination in the majority of the published MRI studies (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994; Thulavath et al., 1995).

Disturbances of ammonia, glutamate and glutamine metabolism in the brain are thought to be of pathogenic importance in CHE (Butterworth et al, 1987; Cooper and Plum, 1987;
Schenker and Brady III, 1994). Glutamate is the major excitatory neurotransmitter in the brain and it is reduced in patients with hepatic encephalopathy (Butterworth et al., 1987). Glutamine, an amino acid formed by amidation of glutamate, plays an important role in ammonia detoxification in the brain, but may itself be neurotoxic (Butterworth et al., 1987), cerebral glutamine levels being considerably elevated in hyperammonaemic conditions such as those seen in patients with CHE (Butterworth et al., 1987). An abnormal population of type II Alzheimer astrocytes has been observed in the basal ganglia in such patients (Norenberg, 1987; Kulisevsky et al., 1992). These cells have large, pale nuclei, prominent nucleoli and are rich in mitochondria, rough endoplasmic reticulum and cytoplasmic vacuolation (Voorhies et al., 1983) and play an important role in ammonia detoxification (Butterworth et al., 1987). They have been suggested as one of the factors responsible for the MRI changes in the globus pallidus of patients with chronic liver failure (Inoue et al., 1991), since the increase in intracellular membrane content in response to elevated ammonia levels may cause $T_1$ shortening. However, the increase in type II Alzheimer cells is not exclusive to the basal ganglia, occurring throughout the brain. Again it would seem unlikely that the presence of these cells in the basal ganglia is the only cause responsible for the hyperintensity seen on $T_1$WSE imaging.

The cerebral metabolism of chronic liver disease patients can be studied in vivo with MR spectroscopy. Utilising proton MRS, it has been previously demonstrated in chapter 8 that elevations in Glx/Cr and reductions in Cho/Cr ratios from voxels localised to the basal ganglia correlate with the severity of the neuropsychiatric impairment. Ammonia itself cannot be detected by proton MRS because of rapid proton exchange with water, but increases in signal from the Glx region of the spectrum reflects ammonia incorporation into glutamine.
A reduction in cerebral choline has been observed in all published MRS series of CHE patients (Chamuleau et al., 1991a; Kreis et al., 1991; Kreis et al., 1992; Ross et al., 1992; Ross et al., 1994). The relative contribution of choline containing compounds to the choline MR peak is not well documented, although cell membrane precursors such as phosphocholine (PC) are major components of this resonance (Miller, 1991). A reduction in the cerebral choline resonance in CHE probably reflects altered cerebral phospholipid metabolism (Miller, 1991). The function of blood brain barrier transport mechanisms may be altered in CHE (James et al., 1978), but animal studies showed that the physical integrity of this barrier remains (Hawkins et al., 1987) despite any changes in cerebral phospholipids.

Few cerebral $^{31}$P MR studies have been undertaken in patients with chronic liver disease, but in chapter 7 it was previously found that there was a reduction in the PME/ATP and PDE/ATP ratios, which correlated with the neuropsychiatric status.

The PME peak is multicomponent and includes contributions from phosphoethanolamine (PE) and PC, intermediates on the pathway of phospholipid membrane synthesis (Ruiz-Cabello and Cohen, 1992), as well as contributions from adenosine monophosphate (AMP) and glycolytic intermediates (Dagnelie et al., 1992). The PDE peak is also multicomponent and contains contributions from glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), intermediates on the pathway of phospholipid membrane breakdown (Ruiz-Cabello and Cohen, 1992), as well as a contribution from endoplasmic reticulum.

The underlying reasons for the reductions that we have reported in the PME/ATP and PDE/ATP ratios in the basal ganglia of patients with chronic liver disease have not been elucidated. The reduced Cho/Cr ratio seen in proton MR spectra may correlate with the observed reductions in PME and PDE found in the phosphorus-31 MR spectra in both in the
current work and the previous study in chapter 7, since PC is a major component of the PME signal and GPC of the PDE signal. This suggests that cerebral phospholipid metabolism is altered in patients with CHE. However, a reduced contribution of PC to the PME resonance may not be sufficient to explain the observed reductions in PME/ATP in this study, but this may also be partially explained by a reduced contribution of glycolytic intermediates to this resonance, as a consequence of decreased glucose utilisation (Jessy et al., 1990; Hilgier et al., 1991). Future studies using carbon-13 MRS and positron emission tomography (PET) may help to clarify these findings.

10.7 Conclusions

It is clear from the present study that there is no correlation between the pallidal hyperintensity seen on T,WSE imaging of the basal ganglia and the changes in metabolite ratios measured in either proton or phosphorus-31 MR spectra of the basal ganglia of patients with chronic liver disease. Whereas the MRS abnormalities reflect alterations in cerebral metabolism in CHE, the results of this study would suggest that the MRI abnormalities of the globus pallidus seen in these patients with chronic liver disease have little to do with the underlying functional abnormalities responsible for the encephalopathy.
10.8 References


# Table 10.1 Neuropsychiatrie status of the patients undergoing MR Imaging and MR Spectroscopy

Mean (range) values

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Age (yr)</th>
<th>Sex ratio (M:F)</th>
<th>Mental state&lt;sup&gt;a&lt;/sup&gt; (0-IV)</th>
<th>Asterixis&lt;sup&gt;b&lt;/sup&gt; (0-4)</th>
<th>EEG&lt;sup&gt;c&lt;/sup&gt; mcf (&gt;=8.9cps)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>NCT A&lt;sup&gt;d&lt;/sup&gt; (15-37)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Blood ammonia µmol/l (11-60)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>PSE sum&lt;sup&gt;e&lt;/sup&gt; (0-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired</td>
<td>44.5</td>
<td>0:4</td>
<td>0</td>
<td>0</td>
<td>9.5</td>
<td>31</td>
<td>134</td>
<td>2.0</td>
</tr>
<tr>
<td>(n=4)</td>
<td>(39-52)</td>
<td></td>
<td></td>
<td></td>
<td>(9-10)</td>
<td>(26-34)</td>
<td>(86-190)</td>
<td>(1-4)</td>
</tr>
<tr>
<td>Subclinical</td>
<td>43.5</td>
<td>3:1</td>
<td>0</td>
<td>0</td>
<td>8.9</td>
<td>44.5</td>
<td>158</td>
<td>3.5</td>
</tr>
<tr>
<td>(n=4)</td>
<td>(21-64)</td>
<td></td>
<td></td>
<td></td>
<td>(8.5-9)</td>
<td>(30-65)</td>
<td>(91-226)</td>
<td>(1-9)</td>
</tr>
<tr>
<td>Overt CHE</td>
<td>49.2</td>
<td>8:5</td>
<td>1-2</td>
<td>2</td>
<td>7.3</td>
<td>59.2</td>
<td>222</td>
<td>10.9</td>
</tr>
<tr>
<td>(n=13)</td>
<td>(32-70)</td>
<td></td>
<td></td>
<td></td>
<td>(0-3)</td>
<td>(23-120)</td>
<td>(98-286)</td>
<td>(6-15)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mental state graded using West Haven criteria (Conn <i>et al</i>, 1977); <sup>b</sup> asterixis grade (Conn <i>et al</i>, 1977); <sup>c</sup>EEG = electroencephalogram; mcf = mean cycle frequency; <sup>d</sup>NCT - Number Connection Test A (Conn, 1977); <sup>e</sup>PSE (Portal Systemic Encephalopathy) Sum (Conn <i>et al</i>, 1977).

+reference ranges
<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Pugh's score*</th>
<th>Child's grade*</th>
<th>Serum bilirubin μmol/L (5-17)*</th>
<th>Plasma albumin (g/L) (35-50)*</th>
<th>Prothrombin time (s) (12-14)*</th>
<th>Serum alkaline phosphatase (u/L) (35-130)*</th>
<th>Serum aspartate transaminase (u/L) (5-40)*</th>
<th>Serum γ glutamyltranspeptidase (u/L) (10-48)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n=4)</td>
<td>6 (5-9)</td>
<td>A-B</td>
<td>85 (10-285)</td>
<td>39 (31-49)</td>
<td>13.5 (13-14)</td>
<td>128 (59-285)</td>
<td>49 (29-70)</td>
<td>130 (48-212)</td>
</tr>
<tr>
<td>Subclinical encephalopathy (n=4)</td>
<td>7 (5-8)</td>
<td>A-B</td>
<td>89 (20-281)</td>
<td>39 (30-52)</td>
<td>14.8 (13-17)</td>
<td>382 (85-589)</td>
<td>122 (34-185)</td>
<td>210 (91-281)</td>
</tr>
<tr>
<td>Overt encephalopathy (n=13)</td>
<td>8 (5-11)</td>
<td>A-C</td>
<td>58 (8-252)</td>
<td>38 (29-45)</td>
<td>15.7 (12-21)</td>
<td>256 (76-946)</td>
<td>69 (34-159)</td>
<td>178 (25-689)</td>
</tr>
</tbody>
</table>

* Degree of functional hepatic impairment (Pugh et al., 1973)
+ reference ranges
Table 10.3  $T_1$-weighted contrast measurements in the globus pallidus

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Contrast measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired</td>
<td>+0.001</td>
</tr>
<tr>
<td>(n=4)</td>
<td>(-0.018 to +0.023)</td>
</tr>
<tr>
<td>Subclinical encephalopathy</td>
<td>+0.044</td>
</tr>
<tr>
<td>(n=4)</td>
<td>(-0.012 to +0.069)</td>
</tr>
<tr>
<td>Overt encephalopathy</td>
<td>+0.133</td>
</tr>
<tr>
<td>(n=13)</td>
<td>(-0.016 to +0.53)</td>
</tr>
</tbody>
</table>

Table 10.4
Comparison of proton MRS metabolite ratios from the basal ganglia in the patient and reference populations

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n=14)</th>
<th>Patient population (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cr</td>
<td>2.07 ± 0.50</td>
<td>2.47 ± 0.91</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.88 ± 0.25</td>
<td>0.51 ± 0.28***</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>0.09 ± 0.03</td>
<td>0.38 ± 0.24***</td>
</tr>
</tbody>
</table>

Values significantly different between patient and reference populations:
*p<0.05; **p<0.01; ***p<0.005.
Table 10.5
Comparison of phosphorus-31 MRS metabolite ratios from the basal ganglia in the patient and reference populations.

Mean (±1 SD) values

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n=15)</th>
<th>Patient population (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME/ATP</td>
<td>1.23 ± 0.35</td>
<td>0.61 ± 0.45**</td>
</tr>
<tr>
<td>PDE/ATP</td>
<td>5.19 ± 2.03</td>
<td>3.04 ± 1.02**</td>
</tr>
<tr>
<td>Pi/ATP</td>
<td>0.91 ± 0.48</td>
<td>0.84 ± 0.26</td>
</tr>
<tr>
<td>PCR/ATP</td>
<td>0.95 ± 0.31</td>
<td>0.99 ± 0.33</td>
</tr>
</tbody>
</table>

Values significantly different between patient and reference populations:
*p<0.05; **p<0.01.
Figure 10.1 a & b

T₁-weighted spin echo images of the basal ganglia (TR 580-760 ms, TE 20 ms) from:
a) a healthy volunteer
b) a patient with overt hepatic encephalopathy

The patient’s image shows bilateral, symmetrical hyperintensity of the globus pallidus.
Figure 10.2
2-D CSI spectrum, (TR 1500ms, TE 130ms) from the basal ganglia of a healthy volunteer.
Cho = choline (3.22ppm), Cr = creatine (3.02ppm),
NAA = N-acetylaspartate (2.02ppm),
Glx = glutamine/glutamate (2.1-2.5ppm).
Figure 10.3
2-D CSI spectra (TR 1500ms, TE 130ms) from the basal ganglia of:
a) a normal volunteer and
b) a patient with overt chronic hepatic encephalopathy.
There is an increase in the Glx/Cr ratio and a decrease in the Cho/Cr ratio in the spectrum from the patient.
Figure 10.4

Localised phosphorus-31 MR spectrum, (TR 1s) from the basal ganglia of a healthy volunteer.
PME = phosphomonoester, Pi = inorganic phosphate, PDE = phosphodiester,
Pcr = phosphocreatine, γ, α, βATP = adenosine triphosphate
Figure 10.5

Phosphorus-31 MR spectra localised to the basal ganglia (TR 1s) from:

a) a healthy volunteer and

b) a patient with overt chronic hepatic encephalopathy. There are decreased PME/ATP and PDE/ATP ratios in the patient's spectrum.
CONCLUSIONS

AND

FUTURE DIRECTIVES
11. CONCLUSIONS AND FUTURE DIRECTIVES

11.1 Hepatic MR Spectroscopy in Chronic Liver Disease

The *in vivo* MRS studies on patients with chronic liver disease demonstrate that spectral abnormalities correlate with hepatic functional decompensation. *In vitro* MRS of liver extracts, obtained at the time of liver transplantation, show that the metabolite changes observed *in vivo* are mainly due to altered phospholipid metabolism with increases in cell membrane precursors and reductions in cell membrane degradation products.

MRS provides direct biochemical information on hepatic metabolic processes and unlike many dynamic liver function tests, it is not blood flow dependent or subject to other extrahepatic influences. The technique may therefore find a role in the assessment of patients for liver transplantation and aid in the timing of the subsequent surgery. Most patients require hepatic imaging during their transplant work-up. MRS may be added on to the end of a standard imaging protocol, provided the appropriate hardware exists, thus answering the criticisms regarding the cost of the technique.

Longitudinal studies are needed in order to assess the sensitivity and specificity of MRS abnormalities in determining patient outcome in chronic liver disease.

11.2 Hepatic MR Spectroscopy Following Liver Transplantation

Allograft rejection is common in patients following orthotopic liver transplantation. Histological analysis of liver biopsy material is the gold standard for diagnosis, but it is subject to sampling error, because the rejection process may affect the liver unevenly. Interpretation of the results also varies between individual observers. *In vivo* MRS results suggest that in patients with chronic ductopenic rejection, spectral abnormalities may be due
to altered phospholipid secretion into bile in this cholestatic condition, where the primary
damage is bile duct directed. Further work is required to establish the precise biochemical
reason responsible for these abnormalities, but the prospect of a non-invasive means of
diagnosing chronic rejection early, before the ultimate decline into liver failure and
subsequent retransplantation may possibly be afforded.

11.3 Future Directives in Hepatic MR Spectroscopy

Technical advances in MRS should allow better resolution of spectra in vivo, particularly
with regard to the PME and PDE resonances. Phosphorus-proton decoupling techniques
may achieve this aim, allowing GPE, GPC, PE and PC resonances to be observed separately.

Phosphorus-31 MRS may be used as a dynamic tool to study hepatic processes such as
hepatic gluconeogenesis, but the development of carbon-13 MRS will allow $^{13}$C labelled
tracers to be used to study carbohydrate and lipid metabolism in patients where liver function
is compromised.

However, in such studies care should be taken not to exceed NRPB guidelines regarding
SAR and power deposition in the area of the body examined.

11.4 Cerebral MR Studies in Chronic Hepatic Encephalopathy

Both $^{31}$P and $^1$H MR spectral abnormalities correlate with neuropsychiatric status. The
abnormalities correct on treatment and may afford the possibility of monitoring patient
responsiveness objectively and also the effectiveness of various treatment regimens. MR
techniques may be used to gain insight into the pathogenesis of the condition.

Future studies should use a combination of $^{31}$P, $^1$H and $^{13}$C MRS to study patients with
chronic liver disease and also those patients following transjugular portasystemic stent
shunting (TIPSS), where encephalopathy develops *de novo*.

On T₁-weighted MR imaging of the brains of patients with chronic liver disease, hyperintensity was observed in the basal ganglia, particularly the globus pallidus. These imaging changes did not correlate with the severity of neuropsychiatric impairment, but these abnormalities were related to the underlying liver function when patients were graded according to the Child-Pugh score. Magnetisation transfer imaging highlighted the basal ganglia, but did not convey any advantage over standard imaging in discerning abnormalities in the brains of patients with chronic liver disease. Future studies should be directed into ascertaining the reasons for such imaging abnormalities, including correlating blood manganese levels with the observed basal ganglial hyperintensity.

When cerebral MRI and MRS abnormalities were observed in the same cohort of patients with chronic liver disease, there was no correlation between the pallidal hyperintensity seen *in vivo* and the spectral changes on both proton and ³¹P MRS.

### 11.5 Future Directives in Cerebral MR Spectroscopy

The development of cerebral ¹³C MRS will allow glutamate metabolism to be studied more closely in the brains of patients with hepatic encephalopathy. Better resolution of the PME and PDE resonances will be achieved using phosphorus-proton decoupling techniques, giving a further handle on cerebral energy metabolism in these patients.

A combination of MRS techniques and positron emission tomography (PET), particularly using ¹¹C-raclopride to study dopaminergic D₂ receptors and ¹¹C-flumazenil to study GABA-ergic systems, will allow the pathogenesis of this complex neuropsychiatric disorder to be unravelled.
Regional Variations in Cerebral Proton Spectroscopy in Patients with Chronic Hepatic Encephalopathy

Simon D. Taylor-Robinson, Janet Sargentoni, Claude D. Marcus, Marsha Y Morgan and David J Bryant

Received 31 August, 1994; Accepted 27 October, 1994

Regional variations in proton magnetic resonance spectroscopy (MRS) were assessed in 26 patients and 14 healthy volunteers using a two dimensional chemical shift imaging technique. Patients were classified as being neuropsychiatrically unimpaired, or as having subclinical or overt chronic hepatic encephalopathy (CHE). Peak area ratios of choline (Cho), glutamine and glutamate (Glx) and N-acetylaspartate (NAA) relative to creatine (Cr) were measured. Significant reductions in mean Cho/Cr and elevations in mean Glx/Cr were observed in the patient population, which correlated with the severity of CHE. There were significant regional variations in these metabolite ratios with the mean Cho/Cr lowest in the occipital cortex and the mean Glx/Cr highest in the basal ganglia. NAA/Cr remained relatively constant in all areas of the brain analysed. The regional variation in the metabolite ratios suggests that spectral information from more than one voxel may be useful in the assessment of patients with CHE.

Key words: Chronic hepatic encephalopathy; regional variation; brain; proton magnetic resonance spectroscopy.

INTRODUCTION

Magnetic resonance spectroscopy (MRS) allows the study of cerebral metabolism in vivo. Phosphorus-31 MRS provides information on cerebral phospholipids, sugar phosphates, phosphocreatine and ATP (Coutts et al., 1989), while proton MRS can be utilised to provide information on brain metabolites such as choline, creatine, N-acetyl aspartate, myoinositol, glutamine and glutamate (Ross et al., 1992).

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A number of proton MRS studies of the brain have been undertaken in cirrhotic patients with chronic hepatic encephalopathy (CHE). These show a reduction in the choline (Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) and myoinositol resonances (Kreis et al., 1990; Bruhn et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) and an increase in the composite glutamine/glutamate resonances (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) obtained from single voxels containing cerebral cortex. We were also interested in information from the basal ganglia since a proportion of patients with CHE display extrapyramidal abnormalities. Furthermore, MR imaging studies have shown areas of high signal from this region of the brain in these patients (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992). In this study we describe the use of a two dimensional chemical shift imaging (2-D CSI) technique to assess regional variations in proton MR spectra in patients with biopsy-proven cirrhosis and relate any changes observed to the patients' neuropsychiatric status.

MATERIALS AND METHODS

Thirty patients with biopsy-proven cirrhosis of varying functional severity and a reference population of 14 healthy volunteers were evaluated for proton MRS study. Spectroscopic information on four patients was unavailable owing to subject movement, claustrophobia or an inability to lie flat for the full examination time. The patient population therefore comprised 26 individuals, 14 men and 12 women, of mean (range) age 49 (21-70) years. Seventeen patients had alcohol-related liver disease, four biliary cirrhosis, three postviral cirrhosis, one autoimmune chronic active hepatitis/cirrhosis and one cryptogenic cirrhosis. Functionally, 11 patients were Child's grade A, eight were Child's grade B and seven were Child's grade C (Pugh et al., 1972). All patients had been abstinent from alcohol for a minimum of 3 months and none was receiving psychoactive medication.

The reference population comprised eight healthy men and six healthy women of mean (range) age 49.4 (31-71) years. None drank alcohol in excess of 20 g/day and none was taking regular medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant. Ethical approval was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London (REC 93/4047) and the Royal Free Hospital and School of Medicine, London. All subjects provided written informed consent.

Patient Assessment:

All patients were clinically stable at the time of the MRS study. A full neurological, psychometric and electrophysiological assessment was performed on all patients within 24 hours of the MRS examination. The mental state of each patient was assessed using West Haven criteria (Conn et al., 1977b). A battery of four psychometric tests were employed, under standardised conditions, comprising Number Connection Tests (NCT) A and B (Conn
Proton MRS in chronic hepatic encephalopathy

et al., 1977a), the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). The same observer was used throughout the study. Electroencephalograms (EEG) were performed using conventionally placed electrodes and a mean cycle frequency was obtained. Immediately prior to the MRS examination, blood was drawn for standard biochemical parameters of liver function. Blood ammonia concentrations were also measured using a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co. Ltd. Kyoto, Japan) (Quero et al., 1993). These data were used to calculate a PSE sum (Conn et al., 1977b) which reflected the severity of the encephalopathy.

The patient population was classified into three groups on the basis of the psychometric analysis, EEG mean cycle frequency and the clinical assessment (Table 1). Five individuals were classified as being neuropsychiatically unimpaired; they had no history of CHE and showed no neuropsychiatric abnormalities when assessed. Ten individuals were classified as having subclinical hepatic encephalopathy; they had no history of CHE and were clinically normal on neurological examination, but showed slowing of their EEG mean cycle frequency and/or impaired performance in at least two of the four psychometric tests employed. One patient in this group was on maintenance treatment with the non-absorbable disaccharide, lactitol (Morgan et al., 1989). The remaining 11 patients either had untreated overt CHE (n=2) or else gave a history of overt CHE requiring long-term maintenance treatment with a non-absorbable disaccharide: (n=9). Five of these patients displayed a mild resting tremor, but there were no other neurological abnormalities.

<table>
<thead>
<tr>
<th>Table 1. Neuropsychiatric status of the patients undergoing proton MRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range) values</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Neuropsychiatric status</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Unimpaired (n=5)</td>
</tr>
<tr>
<td>Subclinical (n=10)</td>
</tr>
<tr>
<td>Overt encephalopathy (n=11)</td>
</tr>
</tbody>
</table>

*Mental state graded using West Haven criteria (Conn et al., 1977b); *Asterix grade (Conn et al., 1977b); *EEG = electroencephalogram; mcf = mean cycle frequency; *NCT - Number Connection Test A (Conn et al., 1977a); *PSE (Portal Systemic Encephalopathy) sum (Conn et al., 1977b).

MR Methods:

Cerebral proton MR spectra were obtained using a Picker prototype spectroscopy system (Picker International, Cleveland, Ohio), based on a whole body magnet (Oxford Magnet Technology, Oxford, UK), operating at 1.5 Tesla. An enveloping quadrature transmit/receive coil tuned to 64MHz, was employed for all examinations.

T1-weighted axial images were acquired in order to position a 2cm transverse slice at the level of the basal ganglia. A 2-D CSI technique was used to obtain spectra from multiple
contiguous voxels covering all the brain in the selected slice. CSI spectral acquisition consisted of a 1331 - 180° spin echo; a 1331 composite pulse for water suppression, with a 90° excitation at the N-acetylaspartate (NAA) resonance, (intra-pulse spacing 2.1ms) a slice selective 180° and phase encoding in the two in-plane directions. The sequence acquisition parameters were TR 1500ms, TE 130ms and 32 phase-encoding steps in each direction giving 1024 averages in 26 minutes. The CSI resolution was 20mm x 10mm x 20mm to give a nominal voxel size of 4cc. In addition, a non-selective inversion pulse preceded each data acquisition, TI 150ms, to reduce the fat signal from the surface voxels and consequent bleeding into neighbouring voxels. Shimming was performed on the water signal from the slice, typically achieving a line-width at half-height of 5 Hz. The total examination time was approximately 90 minutes.

**Data Processing:**

The 2-D CSI spectra were processed with an exponential filter of 120ms (2.7 Hz) in the time domain and cosine filtering in each spatial direction. Data were zero filled to 2048 points in the time domain, prior to Fourier transformation. All spectra were manually phased. Previous studies (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992) have focused on single voxels which contained variable amounts of cerebral cortex. We were also interested in information from the basal ganglia since imaging studies have shown areas of high signal from this region of the brain (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992). Therefore, spectra localised to the basal ganglia, the occipital cortex and temporal cortex were analysed in every case; each voxel was selected by an observer, blinded to the clinical status of all subjects. A knowledge-based algorithm (Saeed, 1993) was used both for removal of the water peak residuum and for baseline flattening. As cerebral creatine has been reported not to vary significantly in patients with CHE, using MR methods (Kreis et al., 1991), this resonance was chosen as an internal reference standard. Peak area ratios of NAA, Cho and Glx relative to Cr were measured by another blinded observer, using the NMRl® spectral processing program (New Methods Research, Inc, E. Syracuse, N.Y.) on a SUN SPARCstation 10 (Sun Microsystems, Inc, Mountain View, C.A.).

**Statistical Analysis:**

Since the data were not normally distributed, non-parametric tests were used for all statistical analyses. Overall regional variations in metabolite ratios were assessed using a Friedman analysis of variance, while comparisons between individual regions of the brain were made using Wilcoxon rank correlations with a Bonferroni correction. Values for the metabolite ratios in the patient and reference populations were compared using the Mann Whitney U test. Comparisons between the patient subgroups were assessed using the Kruskal-Wallis test, while comparisons between the individual patient subgroups and the reference population were made using the Mann Whitney U test with a Bonferroni correction where necessary. In all cases, a p value of <0.05 was considered significant.
RESULTS

A typical cerebral proton MR spectrum (Figure 1) contains resonances which can be assigned to the methyl moieties of choline (Cho) at 3.22 ppm, creatine (Cr) at 3.02 ppm and N-acetyl aspartate (NAA) at 2.02 ppm. The chemical shift of these resonances varied by less than 0.04 ppm in the areas of the brain analysed.

The region of the spectrum between 2.1 and 2.5 ppm includes contributions from both glutamine and glutamate. These resonances have strong homonuclear coupling and are complex multiplet structures which are not easily separated at 1.5T. This composite resonance is referred to as Glx (Kreis et al., 1991). In aqueous solution, the component Glx resonances are largely refocussed at an echo time of 130 ms and have the appearance of a single, broad Gaussian-type peak with the sequences used (Bryant et al., 1994, Taylor-Robinson et al., 1994). Such appearances are the same as those seen in the spectra in vivo (Figure 1) and therefore for the work described in this paper, we have attempted to quantify the resolved spectral lines (NAA, Cr, Cho and Glx) with Gaussian curve-fitting. Other bell-shaped functions, such as Lorentzian, were also investigated, but Gaussian fitting gave the best results in terms of fit and reproducible ratios. A myoinositol resonance is not evident, because this region of the spectrum is only minimally excited with the sequences used in this study.

Figure 1: 3-D CSI spectrum, (TR 1500 ms, TE 130 ms) from the basal ganglia of a healthy volunteer. Cho=choline (3.22 ppm); Cr=creatine (3.02 ppm); NAA=N-acetylaspartate (2.02 ppm); Glx=glutamine/glutamate (2.1-2.5 ppm).
There were significant regional variations in the Cho/Cr and Glx/Cr metabolite ratios in each subject (p<0.05). The NAA/Cr showed no significant regional variation in any individual (Table 2).

In healthy volunteers the mean Cho/Cr was lowest in the occipital cortex and the mean Glx/Cr lowest in the basal ganglia. The NAA/Cr was relatively constant in all the voxels examined (Table 2).

In the patient population the mean Cho/Cr was also lowest in the occipital cortex (Figure 2) and NAA/Cr was relatively constant in all three areas of the brain (Table 2). However, in contrast to the findings in healthy individuals, the mean Glx/Cr was highest in the basal ganglia (Table 2).

Overall, the mean Glx/Cr was significantly higher and the mean Cho/Cr significantly lower in the patient population than in the reference population in all three areas of the brain studied (Table 2). There were no significant differences in NAA/Cr between patients and healthy volunteers (Table 2).

**Table 2. Comparison of metabolite ratios in the patient and reference populations.**

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n=14)</th>
<th>Total patient population (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>2.07 ± 0.50</td>
<td>2.11 ± 0.45</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.88 ± 0.25</td>
<td>0.89 ± 0.28</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>0.09 ± 0.03</td>
<td>0.13 ± 0.13</td>
</tr>
</tbody>
</table>

Reference population
Values significantly different between occipital cortex and other regions, * p<0.05.
Values significantly different between the basal ganglia and other regions, * p<0.05.

Patient population
Values significantly different between the occipital cortex and other regions, * p<0.05.
Values significantly different between the basal ganglia and the other regions, * p<0.05.
Values significantly different between patient and reference populations
* p<0.05; ** p<0.01; *** p<0.005; **** p<0.0005.

The reduction in Cho/Cr and elevation in mean Glx/Cr observed in the patient population reflected the degree of neuropsychiatric impairment (Figure 3, Table 3). Patients who were classified as neuropsychiatrically unimpaired had essentially normal spectra (Figure 3). Patients with overt CHE had a significantly lower mean Cho/Cr than healthy individuals, in all areas of the brain studied (Table 3). The mean Glx/Cr in these patients was significantly higher than both the healthy volunteers and the neuropsychiatrically unimpaired patients (Table 3). The elevation in Glx/Cr was greatest in the basal ganglia (p=0.005) (Table 3). Patients with subclinical hepatic encephalopathy had less pronounced abnormalities in Cho/Cr and Glx/Cr than patients with overt CHE, but these changes were not significantly different from the other two patient groups (Table 3).
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There were no significant correlations between the patients' spectral abnormalities and any of the individual parameters measured: liver function tests, Child's grading, psychometric performance, EEG mean cycle frequency, blood ammonia concentrations or the PSE sum.

Table 3. Comparison of regional metabolite ratios in the patient subgroups, graded according to neuropsychiatric status

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>NAA/Cr</th>
<th>Cho/Cr</th>
<th>Glx/Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>Temporal cortex</td>
<td>Occipital cortex</td>
</tr>
<tr>
<td>Unimpaired (n=5)</td>
<td>1.97 ± 0.37</td>
<td>2.19 ± 0.48</td>
<td>2.52 ± 0.34</td>
</tr>
<tr>
<td>Subclinical encephalopathy (n=10)</td>
<td>2.27 ± 0.37</td>
<td>2.09 ± 0.48</td>
<td>2.37 ± 0.79</td>
</tr>
<tr>
<td>Overt encephalopathy (n=11)</td>
<td>2.53 ± 0.83</td>
<td>2.18 ± 0.75</td>
<td>2.29 ± 0.63</td>
</tr>
</tbody>
</table>

*reference range
Values significantly different between reference and patient populations: *p<0.05; **p<0.01; ***p<0.005.
Values significantly different between neuropsychiatically unimpaired patients and the other patient groups, t-p<0.05.

Figure 2. 3-D CSI spectra (TR 1500 ms, TE 130 ms) from a) the basal ganglia, b) the temporal cortex, c) the occipital cortex of a patient with overt chronic hepatic encephalopathy. There is a marked reduction in the Cho/Cr ratio in the occipital cortex.
Figure 3. 3-D CSI spectra (TR 1500 ms, TE 130 ms) from the basal ganglia of a) a normal volunteer, b) a neuropsychiatrically unimpaired patient, c) a patient with subclinical chronic hepatic encephalopathy, d) a patient with overt chronic hepatic encephalopathy. There is an increase in the Glx/Cr ratio and a decrease in the Cho/Cr ratio with increasing neuropsychiatric impairment.
DISCUSSION

In the present study, significant regional variations in cerebral Glx/Cr and Cho/Cr metabolite ratios were observed in proton MR spectra in all subjects studied. A multi-voxel technique was employed to study regional variations. The binomial 1331 solvent suppression method we used is known to be tolerant of radiofrequency (rf) amplitude (Hore, 1983). However, analysis of the spectra in the regions of interest have demonstrated that resonances do not vary significantly in relationship to this rf variation. The observed proton resonances are partially saturated to a variety of extents and this does not vary significantly between voxels. In addition resonances such as myoinositol, which are close to the unwanted water resonances, can be severely attenuated; the extent depending upon their $T_1$ and $T_2$. Sequences were employed in this study which give a 90° excitation at the NAA resonance, ~200 Hz from water at 1.5T, hence an absence of myoinositol and a higher NAA/Cr than sequences involving CHESS suppression techniques (Kreis et al., 1991; Kreis et al., 1992).

In healthy volunteers the mean Cho/Cr was lowest in the occipital cortex and the mean Glx/Cr lowest in the basal ganglia. The NAA/Cr remained relatively constant throughout the brain. Variation in cerebral metabolite concentrations have been observed in healthy individuals previously (Frahm et al., 1989; Kreis et al., 1993). The results differ depending on the percentage distribution of white and grey matter in the voxels chosen. Thus, Frahm and colleagues (Frahm et al., 1989) studied voxels in the insular area containing mainly grey matter, the occipital area containing mainly white matter, the thalamus and the cerebellum. They assumed a cerebral total creatine concentration of 10mM and calculated the concentration of choline in 14 healthy volunteers to be lowest in the insular area. The thalamus was observed to have the highest concentration of choline. Kreis and co-workers (Kreis et al., 1993) looked at voxels containing mostly white matter from the parietal cortex and mostly grey matter from the occipital cortex in 22 healthy subjects. They observed a lower concentration of choline in the occipital voxels.

Details of regional variations in glutamine/glutamate were not provided in either study, but variations in NAA concentrations were observed in the voxels selected in both studies. Frahm and colleagues (Frahm et al., 1989) found NAA to be lowest in thalamus and cerebellum. Kreis and co-workers (Kreis et al., 1993) calculated the NAA and creatine concentrations to be highest in voxels from the occipital cortex and lowest in voxels from the parietal cortex.

Our results are not directly comparable to other studies, because the areas of the brain studied, the size of voxels selected and the MR sequences used were different. In our total patient population, there was a significant reduction in mean Cho/Cr and a significant elevation in mean Glx/Cr in all regions of the brain when compared to the healthy volunteers. The magnitude of these changes differed significantly in each part of the brain analysed. There were no significant regional variations in NAA/Cr in the total patient group and no significant differences in the magnitude of this ratio from healthy volunteers.

In the patient population, the regional variations in Cho/Cr in the three brain areas studied mirrored the pattern observed in the reference population, while the regional
variation in Glx/Cr was significantly altered. The highest values for this metabolite ratio were observed in the basal ganglia, which was the area where the lowest Glx/Cr values were observed in healthy individuals. Overall the patients showed significantly increased Glx/Cr and significantly reduced Cho/Cr in all brain areas studied. These changes reflected the patients' neuropsychiatric status, to a degree, but there was considerable overlap between patient subgroups. Thus, Glx/Cr values clearly distinguished the patients with overt CHE from those who were neuropsychiatrically unimpaired, but not from those with subclinical impairment. However, it is noteworthy that patients with no neuropsychiatric impairment had essentially normal spectra. It should also be noted that the majority of patients with overt CHE in the present study were on long term maintenance treatment for their encephalopathy and that this may have attenuated the cerebral findings. The distinction between the groups may have been more pronounced if these patients had been studied in their untreated state, but such patients are rarely encountered at a time when they would be sufficiently stable to allow MRS examinations. Furthermore, it would be unethical to stop treatment in patients once stable.

At 1.5T, the glutamine MR resonance is difficult to separate from the glutamate resonance. In the published studies (Kreis et al., 1990; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992), there is agreement that the composite glutamine/glutamate peak is elevated in patients with CHE.

Abnormalities in cerebral glutamine/glutamate and ammonia metabolism are considered to be of pathogenic importance in CHE (Butterworth et al., 1987). Glutamate is the major excitatory neurotransmitter in the brain and it is reduced in patients with CHE (Butterworth et al., 1987). Glutamine, an amino acid formed by amidation of glutamate, plays an important role in ammonia detoxification in the brain, but may itself be neurotoxic (Butterworth et al., 1987). Cerebral glutamine levels are considerably elevated in hyperammonaemic states such as CHE (Butterworth et al., 1987). In animals with encephalopathy, inhibition of cerebral glutamine synthetase and hence reduction in cerebral glutamine concentrations, results in an improvement in the condition (Hawkins et al., 1993). Similarly in man, treatment of hepatic encephalopathy is aimed at reducing circulating ammonia concentrations and hence by extrapolation cerebral glutamine concentrations (Ferenci, 1991). Ammonia cannot be detected by proton spectroscopy because of rapid proton exchange with water. However, changes observed in the glutamine/glutamate region of the spectrum in chronic CHE may reflect ammonia incorporation into glutamine. Blood ammonia levels are not always elevated in these patients (Stahl, 1963) and do not necessarily correlate with symptoms (Fischer, 1992). The condition is heterogeneous and changes in other implicated toxins such as the mercaptans (Zieve et al., 1974) and in different neurotransmitters, such as serotonin (Hawkins et al., 1987), dopamine (Morgan et al., 1980) or norepinephrine (Fischer, 1992) which are not assessable by MRS methods, may be of greater or lesser importance in individual patients.

There is little data on regional variations in Glx in CHE because single voxel techniques have been used in previous studies (Kreis et al., 1990; Bruhn et al., 1991; Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992). However, Bruhn and colleagues (Bruhn et al., 1991) found that in two-thirds of 22 patients studied, Glx was
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generally higher in grey matter than white matter.

In our study, there were regional variations of mean Glx/Cr in the patient population which were most significantly different from the reference population in the basal ganglia (deep grey matter). On T1-weighted MR imaging increased signal is observed in the basal ganglia of such patients (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1992). The reasons behind this hyperintensity are not fully understood, but may represent an accumulation of Alzheimer type II astrocytes (Norenberg, 1987), which are the principal site of glutamate conversion to glutamine in hyperammonaemia (Butterworth et al., 1987). The regional differences observed in Glx/Cr may therefore be dependent on astrocyte function.

A reduction in cerebral choline has been observed in all published MRS series of CHE patients (Chamuleau et al., 1991; Kreis et al., 1991; Kreis et al., 1992, Ross et al., 1992), but regional variations in choline have not been documented previously. Ross and colleagues (Kreis et al., 1991) made the point that in healthy volunteers, Cho/Cr varies considerably between grey and white matter, making this metabolite ratio susceptible to any changes in the grey/white matter distribution in any single voxel examined. Such changes in voxel composition could contribute to the regional variations of Cho/Cr we noted in the patient and reference population and to some of the discrepancies we observed between individuals in each of our patient subgroups. Despite a scatter in results, the reduction in mean Cho/Cr in the patient population in our study appeared to be correlated with neuropsychiatric status.

The relative contribution of choline containing compounds to the choline MR peak is incompletely understood, although cell membrane precursors such as phosphocholine are major components of this resonance (Miller, 1991). A reduction in the cerebral choline resonance in CHE may reflect changes in cerebral phospholipid metabolism (Miller, 1991). The function of blood brain barrier transport mechanisms may be altered in CHE (James et al., 1978), but animal studies showed that the physical integrity of this barrier remains (Hawkins et al., 1987) despite any changes in cerebral phospholipids.

Ross and colleagues (Kreis et al., 1990; Kreis et al., 1991; Kreis et al., 1992; Ross et al., 1992) reported an abnormal myoinositol resonance in patients with this condition, in spectra acquired with a single voxel STEAM sequence; TR1500/TE30ms, voxel size 12-27cm3 (Kreis et al., 1991; Kreis et al., 1992). The significance of such findings remains unclear. We were unable to report on changes in the myoinositol resonance, but regional variations in other metabolites were observed in all patients. These changes were not consistent in every patient and this suggests data from a single area of the brain should be treated with caution and that information from a number of areas of the brain should be considered in individual cases. We would, therefore, conclude that a multivoxel MRS technique may be useful in the assessment of patients with chronic hepatic encephalopathy. The magnitude of the changes in Glx/Cr in the basal ganglia suggests that this area of the brain should be included in routine examinations.
ACKNOWLEDGEMENTS

This study was supported by the Department of Health, the Medical Research Council and Picker International. We would like to thank Professor Neil McIntyre and the staff of the University Department of Medicine at the Royal Free Hospital, Professor Humphrey Hodgson and the staff of the Department of Gastroenterology at the Hammersmith Hospital for kindly allowing us to study their patients.

REFERENCES


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MR Imaging of the Basal Ganglia in Chronic Liver Disease: Correlation of $T_1$-weighted and Magnetisation Transfer Contrast Measurements with Liver Dysfunction and Neuropsychiatric Status

Simon D. Taylor-Robinson$^{1,2,3}$, Angela Oatridge$^1$, Joseph V. Hajnal$^1$, Andrew K. Burroughs$^2$, Neil McIntyre$^2$, Nandita M deSouza$^1$

Conventional $T_1$-weighted spin echo ($T_1$WSE) and $T_1$-weighted magnetization transfer (MT) images were obtained in 26 patients with biopsy-proven cirrhosis (nine Child's grade A, 10 Child's grade B and seven Child's grade C). Four subjects showed no evidence of neuropsychiatric impairment on clinical, psychometric and electrophysiological testing, seven showed evidence of subclinical hepatic encephalopathy and 15 were classified as having overt hepatic encephalopathy. Signal intensities of basal ganglia nuclei (head of caudate, putamen, globus pallidus and thalamus) and adjacent brain parenchyma were measured and contrast calculated. On $T_1$WSE imaging, contrast measurements of the globus pallidus were significantly greater in patients with neuropsychiatric dysfunction than in those who were unimpaired ($p<0.05$). This was not observed in the other basal ganglia nuclei. Patients with subclinical and overt hepatic encephalopathy could not be distinguished on the basis of contrast measurements of the globus pallidus or of any other nucleus. $T_1$WSE contrast measurements of the globus pallidus were increased with elevations in blood ammonia levels ($p<0.05$) and with the severity of liver dysfunction, when graded according to the Pugh's score ($p<0.05$). Those patients with the worst liver injury (Child's grade C) had significantly greater $T_1$WSE pallidal contrast measurements ($p<0.05$) than those patients with minimal liver injury (Child's grade A). The patients with intermediate liver damage (Child's grade B) could not be distinguished from the other two groups. While MT imaging highlighted the basal ganglia and showed a correlation between globus pallidus contrast and blood ammonia levels ($p<0.05$), no other relationship between MT contrast measurements and either the degree of hepatic encephalopathy or the severity of liver dysfunction was found.

Key words: Hepatic encephalopathy; magnetic resonance imaging; neuropsychiatric status; basal ganglia.
assessed by the same observer under standardised conditions, using a battery of four tests comprising Number Connection Tests (NCT) A and B (Conn, 1997), the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955) and the Digit Copying subtest of the Kendrick battery (Kendrick et al., 1979). Electroencephalograms (EEG) were performed using conventionally placed electrodes and a mean cycle frequency obtained. Blood ammonia concentrations were measured using a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co, Ltd, Kyoto, Japan) (Quero et al., 1993). These data were used to calculate a PSE (portal systemic encephalopathy) sum (Conn et al., 1977), which reflected the severity of the encephalopathy.

The patients were classified into three groups on the basis of the psychometric analysis, EEG mean cycle frequency and the clinical assessment (Table 1). Four individuals were classified as being neuropsychiatrically unimpaired; they had no history of CHE and showed no neuropsychiatric abnormalities when assessed. Seven individuals were classified as having subclinical hepatic encephalopathy. They had no history of CHE and were clinically normal on examination, but showed slowing of their EEG mean cycle frequency to below the reference range for an alert adult of >8.9 cycles per second (cps) and/or impaired performance (beyond our reference range from normal healthy volunteers) in at least two of the four psychometric tests employed. One patient in this group was on maintenance treatment with the non-absorbable disaccharide, lactitol (Morgan et al., 1989). The remaining 15 patients either had overt untreated CHE (n=1) or else gave a history of overt CHE, which was episodic or persistent, requiring long-term maintenance treatment with the non-absorbable disaccharides, lactulose (n=4) or lactitol (n=10) (Morgan et al., 1989). The mean length of time since the diagnosis of encephalopathy was 43 months (range 3-266 months). Thirteen of these patients displayed a mild rest tremor, but there were no other neurological abnormalities.

Blood was drawn for standard biochemical and haematological parameters of liver function and a Pugh's score (Pugh et al., 1972) and Child's grade (as modified by Pugh) (Pugh et al., 1972), reflecting the severity of hepatic dysfunction were calculated for each subject. Subjects were then classified according to the severity of liver dysfunction (Table 2). Functionally, nine (34.6%) were Child's grade A, 10 (38.5%) Child's grade B and seven (26.9%) Child's grade C.
INTRODUCTION

Previous magnetic resonance imaging (MRI) studies of the brain in patients with chronic liver disease have demonstrated high signal intensity in the basal ganglia, particularly in the globus pallidus (Brunberg et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994). These observations have been correlated with indicators either of liver dysfunction or chronic hepatic encephalopathy (CHE). However, there is no consensus of opinion as to the precise clinical or biochemical factors which correlate with the MRI appearances. Differences may have arisen between studies because of heterogeneity of patient populations, differences in classification of neuropsychiatric status and variations in the timing of neuropsychiatric assessments with respect to the MRI examination.

The aims of this study were to measure the changes observed on MRI of the basal ganglia in chronic liver disease and to correlate the quantitated signal intensities with the clinical indices of the patients' neuropsychiatric status and the functional severity of their liver disease. Some of the previous studies have not quantified change in signal intensity. We therefore used contrast measurements as a continuous variable in order to assess the relationship between signal intensity in the basal ganglia and both the neuropsychiatric status and indices of hepatic impairment. In order to increase the conspicuity of the deep grey matter, $T_1$W magnetization transfer (MT) images (Hajnal et al., 1992) were used in addition to standard $T_1$W spin echo ($T_1$WSE) techniques.

MATERIALS and METHODS

The study population comprised 26 individuals (15 men and 11 women) of mean age 52 (range 21-70) years with biopsy-proven cirrhosis. Thirteen patients had alcohol-related liver disease, eight biliary cirrhosis, two cryptogenic cirrhosis, two postviral cirrhosis, and one autoimmune chronic active hepatitis/cirrhosis. Twenty-five patients had previously been documented as having oesophageal varices. Twelve individuals had a history of variceal bleeding and six of these had subsequently undergone surgical portacaval shunting. All patients had been abstinent from alcohol for a minimum of 3 months. None of the patients had a history of head injury or cerebrovascular disease and none was receiving psychoactive medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers, ferromagnetic implants or were known to be pregnant. Ethical approval was obtained from the Ethics Committee of the Royal Postgraduate Medical School, London (REC 93/3995). All individuals studied provided written informed consent.

Patient Assessment

A full neurological, psychometric and electrophysiological assessment was performed in all patients on the same day as the MRI study. The mental state of each patient was assessed using West Haven criteria (Conn et al., 1977). Psychometric performance was
Table 2. Relationship of neuropsychiatric status to severity of liver dysfunction

<table>
<thead>
<tr>
<th></th>
<th>Unimpaired</th>
<th>Subclinical encephalopathy</th>
<th>Overt encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=4)</td>
<td>(n=7)</td>
<td>(n=15)</td>
</tr>
<tr>
<td>Pugh's score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (5-9)</td>
<td>7 (5-11)</td>
<td>8 (5-12)</td>
</tr>
<tr>
<td>Child's grade&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A-B</td>
<td>A-C</td>
<td>A-C</td>
</tr>
<tr>
<td>Serum bilirubin µmol/L</td>
<td>86 (10-249)</td>
<td>136 (18-544)</td>
<td>90 (8-616)</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>40 (28-52)</td>
<td>34 (26-44)</td>
<td>37 (26-52)</td>
</tr>
<tr>
<td>Prothrombin time (s)</td>
<td>14 (14)</td>
<td>14.7 (13-17)</td>
<td>17.5 (12-34)</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (u/L)</td>
<td>225 (59-471)</td>
<td>284 (48-793)</td>
<td>223 (69-1553)</td>
</tr>
<tr>
<td>Serum aspartate transaminase (u/L)</td>
<td>64 (22-103)</td>
<td>115 (36-199)</td>
<td>76 (29-236)</td>
</tr>
<tr>
<td>Serum γ-glutamyl-transpeptidase (u/L)</td>
<td>208 (36-364)</td>
<td>141 (26-449)</td>
<td>167 (16-1006)</td>
</tr>
</tbody>
</table>

Values represent Mean with range in parentheses. <sup>a</sup> Degree of functional hepatic impairment (Pugh et al., 1972). + reference ranges.

Statistical Analysis

Non-parametric tests were used for all statistical analyses, because the data were not normally distributed. Comparisons of both $T_J$WSE and MT contrast measurements between the patient subgroups, ranked according to the presence/severity of encephalopathy or the severity of liver dysfunction (Child's grade), were assessed using a non-parametric analysis of variance (Kruskal-Wallis). Comparisons of both $T_J$WSE and MT contrast measurements between individual patient subgroups were made using the Mann Whitney U test with a Bonferroni correction where appropriate. Spearman rank correlations were calculated to determine the relationship between the continuous neuropsychiatric and hepatic variables, including the Pugh's score, and the quantitative measures of basal ganglia signal. In all cases a $p$ value of <0.05 was considered significant.
Table 1. Neuropsychiatric classification of patients undergoing MRI examination

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Unimpaired (n=4)</th>
<th>Subclinical encephalopathy (n=7)</th>
<th>Overt encephalopathy (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>46.5 (40-52)</td>
<td>45.8 (21-64)</td>
<td>56.2 (32-70)</td>
</tr>
<tr>
<td>Sex ratio (M:F)</td>
<td>1:3</td>
<td>4:3</td>
<td>10:5</td>
</tr>
<tr>
<td>Mental state&lt;sup&gt;a&lt;/sup&gt; (0-IV)</td>
<td>0</td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td>Asterixis&lt;sup&gt;b&lt;/sup&gt; (0-IV)</td>
<td>0</td>
<td>0</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>EEG&lt;sup&gt;c&lt;/sup&gt; mcf (28.9 cps)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>9.5 (9-10)</td>
<td>8.9 (7.5-10)</td>
<td>7.1 (4.5-9.5)</td>
</tr>
<tr>
<td>NCT A&lt;sup&gt;d&lt;/sup&gt; (15-37)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>30 (26-34)</td>
<td>48 (37-65)</td>
<td>67 (23-145)</td>
</tr>
<tr>
<td>Blood ammonia µmol/l (11-60)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>151 (91-190)</td>
<td>211 (86-286)</td>
<td>200 (98-286)</td>
</tr>
<tr>
<td>PSE sum&lt;sup&gt;e&lt;/sup&gt; (0-28)</td>
<td>3.0 (1-4)</td>
<td>4.4 (1-6)</td>
<td>12.0 (6-22)</td>
</tr>
</tbody>
</table>

Values represent Mean with range in parentheses. <sup>a</sup>Mental state graded using West Haven criteria (Conn et al., 1977); <sup>b</sup>asterixis grade (Conn et al., 1977); <sup>c</sup>EEG=electroencephalogram; mcf=mean cycle frequency; <sup>d</sup>NCT: Number Connection Test A (Conn, 1977); <sup>e</sup>PSE (Portal Systemic Encephalopathy) sum (Conn et al., 1977). + reference ranges.

Imaging Methods

MRI of the basal ganglia was performed on a 1.0 T Picker Vista HPQ system (Picker International, Cleveland, Ohio). Conventional transverse $T_1$-weighted spin echo ($T_1$WSE) images were obtained using repetition times (TR) 580-760 ms, echo time (TE) 20 ms with a slice thickness of 6 mm, phase resolution 128 x 256 and two excitations. $T_1$-weighted magnetization transfer (MT) images were also obtained with the same sequence parameters, by the addition of a saturating radiofrequency irradiation pulse with a frequency offset of 1000 Hz, amplitude of 11.5 µT and a 56% duty cycle (Hajnal et al., 1992).

Image Analysis

The basal ganglia nuclei (the head of the caudate nucleus, the putamen, the globus pallidus and the thalamus) were visually assessed by one observer who was blinded to the patient's clinical condition, the conspicuity of each nucleus being graded as normal or increased by comparison to the appearance of images from normal volunteers. Signal intensities (SI) of the same nuclei and the adjacent white matter (WM) were measured using scanner resident software. The nucleus to background contrast was then calculated using the formula: $SI_{nucleus} - SI_{WM} / SI_{nucleus} + SI_{WM}$

No signal intensity measurements were made in six of the patients with overt encephalopathy because their MR images were grossly degraded by motion artefact.
Table 3. T₁-weighted contrast measurements in each of the basal ganglia nuclei

<table>
<thead>
<tr>
<th>Neuropsychiatric status (n)</th>
<th>Unimpaired (n=4)</th>
<th>Subclinical encephalopathy (n=7)</th>
<th>Overt encephalopathy (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globus pallidus</td>
<td>+0.001</td>
<td>+0.059*</td>
<td>+0.096*</td>
</tr>
<tr>
<td></td>
<td>(-0.018 to +0.019)</td>
<td>(-0.012 to +0.11)</td>
<td>(-0.016 to +0.53)</td>
</tr>
<tr>
<td>Head of caudate</td>
<td>-0.027</td>
<td>-0.018</td>
<td>-0.024</td>
</tr>
<tr>
<td></td>
<td>(-0.07 to +0.009)</td>
<td>(-0.063 to +0.022)</td>
<td>(-0.073 to +0.041)</td>
</tr>
<tr>
<td>Putamen</td>
<td>-0.023</td>
<td>-0.002</td>
<td>-0.008</td>
</tr>
<tr>
<td></td>
<td>(-0.04 to -0.006)</td>
<td>(-0.043 to +0.025)</td>
<td>(-0.063 to +0.047)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>-0.037</td>
<td>-0.06</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>(-0.07 to -0.02)</td>
<td>(-0.11 to -0.03)</td>
<td>(-0.095 to -0.012)</td>
</tr>
</tbody>
</table>

Values represent Mean with range in parentheses. Significant difference between neuropsychiatrically unimpaired patients and the other two groups: * p<0.05. + reference ranges.

There was no relationship between the T₁WSE contrast measurements and the length of time since individual patients were diagnosed with encephalopathy. Similarly, there was no association between the contrast measurements in the basal ganglia and the EEG mean cycle frequency, psychometric performance or the PSE sum.

There was a significant relationship between the measured contrast in the globus pallidus on T₁WSE imaging and blood ammonia levels (p<0.05). There was also a significant association between the T₁WSE contrast measurements of the globus pallidus and the severity of liver dysfunction, when patients were classified according to Pugh's score (p<0.05). In those patients with minimal liver injury (Child's grade A), the contrast measurements in the globus pallidus were significantly less (p<0.05) than in those patients with the worst liver function (Child's grade C). Patients with intermediate liver damage (Child's grade B) could not be distinguished from the other two groups.

There was no significant relationship between the measured pallidal contrast on T₁WSE imaging and the prothrombin time, serum albumin or standard biochemical liver function tests. Furthermore, there was no correlation between the T₁WSE contrast measurements of the other basal ganglia nuclei and any index of liver dysfunction.

There was also no relationship between contrast measurements of any of the nuclei including the globus pallidus on conventional T₁WSE imaging and the presence of asterixis or rest tremor, the presence of portasystemic shunting and/or oesophageal varices or a history of previous variceal bleeding.

T₁-weighted magnetization transfer (MT) images showed the basal ganglia nuclei with greater conspicuity in normal volunteers than on standard T₁WSE imaging (Figure 2). On visual assessment of our patient group, 14 of the 17 patients noted to have pallidal
RESULTS

On visual assessment of the conventional $T_1$WSE images, there was bilateral, symmetrical hyperintensity of the globus pallidus (Figure 1) in 17 patients (65%) and of the putamen in five patients (19%), compared to normal volunteers. There was a significant difference ($p<0.05$) in the $T_1$WSE contrast measurements of the globus pallidus between the non-encephalopathic individuals and those with subclinical and overt CHE, when the latter two groups were considered both separately and together (Table 3). This difference was not seen in any of the other basal ganglia measured (Table 3). In patients with subclinical encephalopathy, the $T_1$WSE contrast measurements of all the nuclei, including the globus pallidus, were not significantly different from those of patients with overt CHE (Table 3).

![Figure 1A,B](image)

**Figure 1A,B.** $T_1$-weighted spin echo images of the basal ganglia (TR 580-760 ms, TE 20 ms) from A) a healthy volunteer, B) a patient with overt hepatic encephalopathy showing bilateral, symmetrical hyperintensity of the globus pallidus.
Table 4. Magnetization transfer contrast measurements in each of the basal ganglia nuclei

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Unimpaired (n=4)</th>
<th>Subclinical encephalopathy (n=7)</th>
<th>Overt encephalopathy (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globus pallidus</td>
<td>+0.067 (0.013 to +0.11)</td>
<td>+0.131 (0.044 to +0.19)</td>
<td>+0.112 (0.02 to +0.17)</td>
</tr>
<tr>
<td>Head of caudate</td>
<td>+0.065 (0.031 to +0.089)</td>
<td>+0.049 (0.003 to +0.1)</td>
<td>+0.05 (0.001 to +0.077)</td>
</tr>
<tr>
<td>Putamen</td>
<td>+0.063 (0.029 to +0.081)</td>
<td>+0.065 (0.024 to +0.1)</td>
<td>+0.49 (0.038 to +0.074)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>-0.05 (-0.056 to +0.032)</td>
<td>-0.073 (-0.16 to -0.015)</td>
<td>-0.055 (-0.13 to +0.03)</td>
</tr>
</tbody>
</table>

Values represent Mean with range in parentheses. + reference ranges.

DISCUSSION

The globus pallidus appeared bilaterally and symmetrically hyperintense on conventional T1WSE images and showed a significant increase in measured contrast with the presence, but not the severity, of encephalopathy. Pallidal T1WSE contrast measurements were also significantly related to the severity of liver dysfunction. None of these relationships could be demonstrated for any of the other basal ganglia.

On visual assessment, MT imaging highlighted all the basal ganglia nuclei, but none of the MT contrast measurements discriminated between patients graded according to the severity of liver dysfunction or the neuropsychiatric status. However, pallidal MT contrast measurements were significantly associated with elevations in blood ammonia levels.

Pallidal hyperintensity has been noted by previous investigators (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994). On T1-weighted imaging in 56-100% of patients with chronic liver disease. In the majority of these studies (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Kulisevsky et al., 1992; Pujol et al., 1993; Norton et al., 1994) no significant relationship was observed between the increased signal intensity and the patients' neuropsychiatric status at the time of MRI examination. However, our clinical, electrophysiological and neuropsychiatric assessments were performed on the same day as the MRI examination, whereas this was not achieved in other studies. Zeneroli and colleagues (Zeneroli et al., 1991) noted that the majority of patients with encephalopathy had these imaging changes, but no correlation was made between the magnitude of the MRI abnormalities and the degree of neuropsychiatric impairment. In our study, while there was a significant difference in pallidal contrast measurements between neuropsychiatrically normal and encephalopathic patients on T1WSE imaging, no difference could be found between the groups with subclinical and overt encephalopathy.
hyperintensity on conventional $T_1$-WSE imaging, also showed striking bilateral, symmetrical pallidal hyperintensity on MT imaging (Figure 2, Table 4). This exaggeration of the normal pattern was also seen in the putamen in four patients and in the head of the caudate nucleus in two patients. A significant relationship was noted between blood ammonia levels and the MT contrast measurements in the globus pallidus ($p<0.05$). This was not observed in any of the other nuclei. However, MT contrast measurements failed to discriminate between patient groupings when subjects were classified according to the presence or severity of encephalopathy using the West Haven criteria (Table 4), or the degree of liver dysfunction using the Child-Pugh score.

**Figure 2A,B.** $T_1$-weighted magnetization transfer images of the basal ganglia (TR 580-760 ms, TE 20 ms; frequency offset 1000 Hz, amplitude 11.5 μT, 56% duty cycle) from A) a normal volunteer, B) a patient with overt hepatic encephalopathy. All the basal ganglia are highlighted with most striking changes in the globus pallidus.
Gut derived toxins, such as ammonia are implicated in the pathogenesis of CHE (Cooper and Plum, 1987; Schenker and Brady 1994). Elevations in blood ammonia may be consequent to hepatic failure (Kulisevsky et al., 1992) or to portasystemic shunting (Ferenci, 1991). It is interesting to note the correlation between blood ammonia levels and the MRI signal of the globus pallidus on T1-weighted imaging, observed by Kulisevsky and colleagues (Kulisevsky et al., 1992) and on both T1WSE and MT imaging in the current study.

Proton magnetic resonance spectroscopy (MRS) has been used to demonstrate regional variations in cerebral metabolites in patients with chronic hepatic encephalopathy (Taylor-Robinson et al., 1994). Peak area ratios of glutamine and glutamate relative to creatine were most elevated in the basal ganglia. Ammonia cannot be detected by proton MRS because of rapid proton exchange with water. However, changes in the glutamine/glutamate region of the spectrum reflect ammonia incorporation into glutamine. The changes in these metabolites, most obvious in the basal ganglia, may be consequent to regional differences in astrocyte function. The changes observed in the basal ganglia on T1-weighted imaging in patients with CHE may parallel the functional abnormalities observed with proton MRS.

In animal models of hepatic encephalopathy, an abnormal accumulation of type II Alzheimer cells has been demonstrated in the basal ganglia (Norenberg, 1987). These astrocytes play an important role in ammonia detoxification in the brain (Butterworth et al., 1987) and may be responsible for the MRI changes in the globus pallidus of patients with chronic liver failure (Inoue et al., 1991). These abnormal cells have large, pale nuclei, prominent nucleoli and are rich in mitochondria, rough endoplasmic reticulum and cytoplasmic vacuolation (Voorhies et al., 1983). This proliferation of cellular organelles in response to hyperammonaemia may alter signal intensity on T1-weighted images by increasing intracellular membrane content. A limited number of post-mortem studies has been undertaken on patients dying with hepatic failure, who showed cerebral MRI abnormalities during life. Kulisevsky and co-workers (Kulisevsky et al., 1992) reported two patients in whom Alzheimer type II cells were noted in the caudate, putamen and the globus pallidus. In CHE, there may be regional differences in astrocyte function in response to circulating toxins (Norenberg, 1981). However, these astrocytes are not confined to the basal ganglia (Norenberg, 1981) and it would therefore seem unlikely that the presence of these cells are solely responsible for the T1 shortening on T1WSE images.

Pallidal demyelination (Kulisevsky et al., 1992) and lipid droplets (McConnell and Castaldo 1990; Kulisevsky et al., 1992) have also been noted in post-mortem studies. Lipid accumulation has been suggested as the cause of the signal abnormalities in the globus pallidus (Zeneroli et al., 1991) and circulating short chain fatty acids have been implicated in the pathogenesis of hepatic encephalopathy (Chen et al., 1970), but post-mortem studies have only demonstrated small quantities of lipid in the basal ganglia of these patients (Victor et al., 1965).

Oxidative damage resulting from free-radical production has been suggested as the central mechanism in a number of neurodegenerative diseases of the basal ganglia including Huntington's disease and progressive supranuclear palsy (Jenner, 1994). Such oxidative
Pujol and colleagues (Pujol et al., 1991; Pujol et al., 1993) found no correlation between the globus pallidus hyperintensity and the patients' current mental state or EEG findings, but observed a significant correlation with previous episodes of hepatic encephalopathy (Pujol et al., 1993). There was also a significant correlation with the presence of rest tremor. Kulisevsky and colleagues (Kulisevsky et al., 1992) found a relationship between the imaging appearances in the globus pallidus and a history of recurrent hepatic encephalopathy. Dysdiadochokinesis, the presence of primitive reflexes, postural tremor, EEG slowing and performance in certain psychometric tests were also related to pallidal changes by these authors. These results differ from our findings, in that we observed no association between $T_1$WSE contrast measurements and EEG mean cycle frequency, psychometric performance, the PSE sum or the presence of rest tremor. This may be because the patient populations and methods of patient assessment were different.

In our group, on $T_1$WSE imaging, the measured pallidal contrast correlated significantly with the severity of liver dysfunction, as indicated by the Child-Pugh score. There was also a significant relationship with elevations in blood ammonia levels, but not with the presence of portasystemic shunting and/or oesophageal varices or a history of previous variceal bleeding. None of these relationships was observed in the other basal ganglia nuclei.

In contrast, there was no significant relationship between the presence of globus pallidus hyperintensity and either the aetiology or functional severity of liver disease reported in most studies (Brunberg et al., 1991; Inoue et al., 1991; Pujol et al., 1991; Syh et al., 1991; Zeneroli et al., 1991; Kulisevsky et al., 1992), but Pujol and colleagues (Pujol et al., 1993) did find a significant correlation between these MRI findings and the Child-Pugh score. They concluded that MRI abnormalities in these patients are associated with the severity of liver failure, but unlike our study, these authors also found a correlation with previous episodes of variceal bleeding as well as with abnormalities in various biochemical liver function tests.

Kulisevsky and co-workers (Kulisevsky et al., 1992) noted a correlation between the globus pallidus signal and blood ammonia levels. They also observed that the signal was higher in patients with portasystemic shunts. Similarly, Inoue and colleagues observed in their study (Inoue et al., 1991) that the nine subjects who showed globus pallidus hyperintensity all had large portal-systemic collateral vessels, receiving blood from the superior mesenteric vein. However, no signal intensity measurements were made in this series.

The cause of the increased signal intensity in the basal ganglia of CHE patients on $T_1$WSE imaging is uncertain. Reversal of pallidal hyperintensity has been described in patients following liver transplantation (Pujol et al., 1991; Pujol et al., 1993), indicating that these changes are closely linked to hepatic function. However, signal increases in the basal ganglia have also been observed on $T_1$-weighted images in patients with systemic lupus erythematosus (Brunberg et al., 1991), neurofibromatosis (Mirowitz et al., 1989), and those receiving long-term parenteral nutrition (Mirowitz et al., 1991), indicating that these abnormalities are characteristic, but not specific to patients with chronic hepatic failure. Furthermore, Gupta and colleagues (Gupta et al., 1993) reported on three patients with acute...
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We thank Dr Marsha Morgan from the University Department of Medicine at the Royal Free Hospital for helpful advice and Professor Humphrey Hodgson and the staff of the Department of Gastroenterology at the Hammersmith Hospital for kindly allowing us to study their patients. We are grateful to the staff of the Medical Statistics Department, Royal Postgraduate Medical School, for their assistance.

REFERENCES


stress may well occur in CHE, resulting in lipid peroxidation and the observed changes on T₁-weighted imaging. However, the free-radical species involved and the cause of increased free-radical production, have yet to be identified.

The deposition of materials such as melanin, manganese and deoxyhaemoglobin can cause T₁ shortening and this may be responsible for hyperintensity on T₁-weighted images. The results of most post-mortem studies in patients dying of chronic liver failure have not shown increased deposition of these substances in the basal ganglia (Victor et al., 1965; Levy et al., 1989; Kulisevsky et al., 1992). However, Pomier-Layrargues and colleagues (Pomier-Layrargues et al., 1995) found increased manganese concentrations in the globus pallidus of nine patients who died in hepatic coma. They suggested that these abnormalities in the basal ganglia may be responsible for the extrapyramidal symptoms seen in patients with hepatic encephalopathy and the pallidal hyperintensity seen on MRI.

Signal changes other than in the globus pallidus have been reported in CHE. Inoue observed increased signal in portions of the internal capsule (Inoue et al., 1991), while Brunberg noted similar changes in the mesencephalon surrounding the red nucleus, quadrigeminal plate and anterior pituitary (Brunberg et al., 1991). In neither of these studies was the contrast of the basal ganglia quantified in the various clinical groups. Norton and colleagues (Norton et al., 1994) quantified signal differences between patients with CHE and normal controls in both the limbic and the extrapyramidal systems and also the associated myelinated pathways. However, no correlation was made between signal intensity and the severity of encephalopathy or liver dysfunction. On visual assessment of T₁WSE images, we observed hyperintensity in the putamen of five patients, while on similar assessment of MT images both the putamen and the head of the caudate nucleus appeared brighter than normal in a small minority of individuals.

MT imaging has not previously been reported in patients with CHE. This technique highlighted the basal ganglia and improved lesion conspicuity, but MT contrast measurements did not add to the information obtained from T₁WSE images. Furthermore, because in normal subjects, the basal ganglia are hyperintense on MT images, the further increase in signal associated with CHE may be less easily recognised than corresponding changes on T₁-weighted images.

It is obvious that there has been no consensus of opinion as to the precise factors which correlate with the hyperintensity seen in the basal ganglia of patients with chronic liver disease, but our study is the first to correlate T₁WSE imaging contrast measurements with both the severity of liver dysfunction and the presence, although not the severity, of encephalopathy. T₁WSE imaging may act as an indicator of exposure to circulating toxins in CHE, where the pathogenesis is multifactorial and its relationship to the severity of liver disease is complex. Further studies are required to elucidate the cause of the changes in the basal ganglia seen on T₁-weighted imaging. These may include a combination of MRI and MRS to evaluate the functional changes behind our observations.


Cerebral Phosphorus-31 Magnetic Resonance Spectroscopy in Patients with Chronic Hepatic Encephalopathy

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Cerebral phosphorus-31 magnetic resonance spectroscopy was undertaken in 33 patients with biopsy-proven cirrhosis: 6 had no evidence of neuropsychiatric impairment on standard clinical, psychometric and electrophysiological testing; 8 had evidence of subclinical hepatic encephalopathy; and 19 were classified as having overt hepatic encephalopathy. The reference population comprised 15 healthy volunteers. Unlocalized spectra were acquired from the entire head with a 45-degree pulse angle and repetition times of 1 and 5 sec. Spectra localized to the basal ganglia were acquired with a 45-degree pulse angle and a repetition time of 1 sec. Peak area ratios of phosphomonooesters, inorganic phosphate, phosphodiesters and phosphocreatine relative to p-ATP were measured in the spectra acquired. We noted no consistent change in the ratios of inorganic phosphate to ATP and phosphocreatine to ATP. Mean values of the ratios of phosphomonooesters to ATP and phosphodiesters to ATP were significantly lower in the total patient population than in the reference population, and they correlated with the patients' neuropsychiatric status. Thus we found no significant reductions in the mean ratios of phosphomonooesters to ATP and phosphodiesters to ATP in patients who were neuropsychiatically unimpaired, but significant reductions were observed in the mean ratios of phosphomonooesters to ATP and phosphodiesters to ATP in patients with both subclinical and overt hepatic encephalopathy. The most marked reductions in these metabolite ratios were observed in patients with overt encephalopathy.

SUBJECTS AND METHODS

The patient population comprised 33 individuals (18 men and 15 women) with biopsy-proven cirrhosis (mean age, 52.2 yr; range, 32 to 67 yr). Twenty-three patients (69.7%) had alcohol-related liver disease, five (15.2%) had cryptogenic cirrhosis, three (9.0%) had biliary cirrhosis and two (6.1%) had autoimmune CAH/cirrhosis. Functionally, four (12.1%) were of Child grade A, 20 (60.6%) were Child grade B and nine
Table 1. Neuropsychiatric status of the patients undergoing 31P MRS

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>Age (yr)*</th>
<th>Sex ratio (M/F)</th>
<th>Child class C (%)</th>
<th>Mental state (0-IV)*</th>
<th>Asterixis (0-IV)**</th>
<th>EEG mean cycle frequency***</th>
<th>NCT A****</th>
<th>Blood ammonia (µmol/L)*</th>
<th>PSE sum**</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n = 6)</td>
<td>42.8</td>
<td>36-51</td>
<td>1:5</td>
<td>0</td>
<td>0</td>
<td>0.99 (9-11)</td>
<td>27 (17-33)</td>
<td>191 (86-286)</td>
<td>3.2 (2-4)</td>
<td></td>
</tr>
<tr>
<td>Subclinical encephalopathy (n = 8)</td>
<td>50.0</td>
<td>39-60</td>
<td>5:3</td>
<td>16.7</td>
<td>0</td>
<td>9.1 (7.5-10)</td>
<td>46 (26-78)</td>
<td>135 (78-217)</td>
<td>4.5 (2-8)</td>
<td></td>
</tr>
<tr>
<td>Overt encephalopathy (n = 8)</td>
<td>56.0</td>
<td>32-67</td>
<td>13:6</td>
<td>21.0</td>
<td>I-II</td>
<td>7.8 (6-10)</td>
<td>50 (24-180)</td>
<td>240 (100-286)</td>
<td>11.0 (3-16)</td>
<td></td>
</tr>
</tbody>
</table>

*Data expressed as mean (range).
**Graded according to West Haven criteria (9).
***Reference range, ≥6.9 cps.
**Number-connection test A (reference range, 15 to 37) (10).
*Reference range, 11 to 60 µmol/L.
*Reference range, 0 to 25.

(27.3%) were Child grade C (8). All patients had abstained from alcohol for at least 3 mo, and none was taking psychoactive medication.

The reference population comprised 15 healthy volunteers (7 men and 8 women) (mean age, 40.8 yr; range, 21 to 67 yr); none drank alcohol in excess of 20 gm/day and none was taking regular medication.

Individuals were excluded from the study if they were claustrophobic, had cardiac pacemakers or ferromagnetic implants or were known to be pregnant.

All individuals studied provided written informed consent. Permission for this study was obtained from the ethics committees of the Royal Postgraduate Medical School, London (study approval no. REC 93/4047) and the Royal Free Hospital and School of Medicine, London.

Patient Assessment. All patients underwent full neurological, psychometric and electrophysiological assessment on the morning of MRS study. Patients were examined clinically, and their mental state was assessed according to West Haven criteria (9). Psychometric performance was assessed under standardized conditions by the same observer, using a battery of four tests: number-connection tests A and B (10), the digit symbol subtest of the Wechsler Adult Intelligence Scale (11) and the digit-copying subtest of the Kendrick battery (12). EEGs were performed with conventionally placed electrodes, and a mean cycle frequency was obtained. The blood ammonia concentration was measured in each subject immediately before MRS examination with a Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co., Ltd., Kyoto, Japan) (13). A PSE sum (9) was then calculated for each subject.

The patient population was classified into three groups on the basis of this assessment (Table 1). Six individuals had no history of hepatic encephalopathy and showed no neuropsychiatric abnormalities on assessment; they were classified as being neuropsychiatrically unimpaired. Eight individuals had no history of overt hepatic encephalopathy and were clinically normal on examination but showed slowing of their EEG mean cycle frequency to below the reference range for an alert adult (e.g., 8.9 cycles or more per second, impaired performance in at least two of the four psychometric tests employed or both.

These patients were classified as having subclinical hepatic encephalopathy; three patients in this group were on maintenance treatment with lactitol (14). The remaining 19 patients had overt untreated hepatic encephalopathy (n = 2) or gave a history of overt hepatic encephalopathy requiring long-term maintenance treatment (n = 17).

NMR Methods. In vivo cerebral 31P MR spectra were obtained with a Picker prototype spectroscopy system (Picker International, Cleveland, OH) based on a whole-body magnet (Oxford Magnet Technology, Oxford, UK), operating at 1.5 T (15). An enveloping saddle-shaped transmitter coil and a separate saddle-shaped receiver coil, into which each subject's head could be comfortably positioned, were employed for all examinations. Both coils were double-tuned for phosphorus and proton frequencies, at 25.9 MHz and 64 MHz, respectively. The proton signal was used for shimming and to acquire T1-weighted axial imaging for verification of spectral localization.

Unlocalized spectra were acquired from the entire head in all 48 subjects, with TRs of 1 and 5 sec. Data were accumulated for 128 averages at 1 sec, and 64 averages were collected at 5 sec; examinations took 2 min or 5 min, respectively.

Localization was achieved by use of a 4D-CSI technique (15). A total of 512 voxels in a cubic array, 8 x 8 x 8 in dimensions, covering the whole head, was acquired, with each voxel containing a conventional MR absorption spectrum. A total of 2,048 averages was acquired for the 4D-CSI examination at a TR of 1 sec; a nominal spatial resolution of (3 cm)3 was obtained in approximately 34 min.

For both unlocalized and localized examinations, a 45-degree radio frequency pulse was employed. The pulse was calibrated with an external pickup loop, which monitored the radio frequency field directly. The variations in loading on the transmitter system between phantom calibration studies and in vivo human studies were corrected for with this procedure.

The choice of a 45-degree radio frequency pulse is commensurate with improved spectral appearances or SNR in instances where repetition time is sufficiently low that resonances are partially saturated (16). The full protocol for this study, as described above, took approximately 120 min.

Most patients also underwent a formal imaging examination with a Picker HPQ Vista system operating at 1.0 T. Standard T1-weighted (TR 760 ms, TE 20 ms) and T2-weighted (TR 2500 ms, TE 20/80 ms) spin-echo techniques were used. T1-weighted magnetization transfer imaging sequences (TR 760 ms, TE 20 ms) were obtained through the basal ganglia with a frequency offset of 1,000 Hz.

Data Processing. The unlocalized spectra were processed with exponential difference filters of 1 and 60 ms and were manually phased. The 1-ms exponential filter was employed to remove the broader resonance that is usually attributed to bone and lipid components. SNR was improved by line broadening with the 60-ms exponential (or 5-Hz Lorentzian) filter. The localized spectra were processed with a cosine filter in all three spatial directions and a 30 ms exponential filter and were...
also manually phased. The filtering of the broad resonance was unnecessary because of the introduction of the spatial localizing field gradient pulses. The baseline roll, resulting from this delay in data acquisition while phase-encoding gradients were applied in the 4D-CSI sequences, was removed with a knowledge-based algorithm (17). A manual baseline correction was used, where necessary, and peak areas ratios of PME, Pi, PDE and PCR relative to β-ATP and PCr/Pi were measured by a blinded observer using the NMRI spectral processing program (New Methods Research, Inc., East Syracuse, NY) on a SUN SPARCstation 1+ (Sun Microsystems, Inc., Mountain View, CA). The data were fitted to inverse polynomial functions.

The 95% confidence intervals for the individual metabolite ratios in the healthy volunteers were used to define the reference range. Values outside this range were considered abnormal. Because the data were not normally distributed, nonparametric tests were used for all statistical analyses. Values for the metabolite ratios in the patient and reference populations were compared by means of the Mann-Whitney U test. Comparisons between the patient subgroups were carried out with a nonparametric ANOVA (Kruskal-Wallis). Comparisons between individual patient subgroups and the reference population were made with the Mann-Whitney U test, with a Bonferroni's correction where appropriate. In all cases, a p value of less than 0.05 was considered significant.

RESULTS

The cerebral 31P MR spectrum from a healthy volunteer contains at least seven resonances (18), which can be attributed to PME, Pi, PDE, PCR, γ-ATP, α-ATP and β-ATP (Fig. 1). The PME and PDE peaks are multi-component, the α-ATP peak contains contributions from α-ADP and NADH and the γ-ATP peak contains contributions from β-ADP.

Unlocalized spectra were obtained from all subjects. The protocol was not completed by 10 patients and 2 volunteers, because of subject movement or discomfort; therefore localized spectra were obtained in 23 patients and 13 volunteers.

We chose to analyze spectra localized to the basal ganglia because several of our patient cohort showed some minor extrapyramidal signs of parkinsonian type and because hyperintensity of the globus pallidus has been observed on T1-weighted images in a high percentage of patients with chronic liver disease (19). Magnetization transfer T1-weighted images at 1.0 T highlighted the increased signal in the basal ganglia in these patients (Fig. 2). Only the unlocalized spectra and those spectra localized to the basal ganglia were used for further comparisons.

We found no additional resonances noted in the patients' spectra (Figs. 1 and 2), but there were significant changes in certain metabolite ratios. The mean (±1 S.D.) ratio of PME to ATP was significantly reduced in the patient population, compared with that of the reference population, in both unlocalized spectra and in spectra localized to the basal ganglia (Table 2). Similarly, significant reductions were observed in the mean ratio of PDE to ATP in the patient population in both unlocalized and localized spectra, although the magnitude of the reduction was smaller (Table 2).

The changes in the mean ratios of PME to ATP and of PDE to ATP correlated overall with neuropsychiatric status (Table 3). No significant reductions in the mean ratios of PME to ATP or PDE to ATP were observed in the patients who were neuropsychiatrically unimpaired, but significant reductions in the mean values for these metabolite ratios were observed in the presence of neuropsychiatric impairment.

Significant reductions in the mean ratio of PME to ATP were observed in patients with subclinical encephalopathy in both unlocalized and localized spectra (Table 3). No significant difference was observed in the mean ratio of PDE to ATP in unlocalized spectra acquired with TR 1 sec in these patients, although significant reductions were observed in the mean ratio of PDE to ATP in unlocalized spectra acquired with TR 5 sec in these patients, although significant reductions were observed in the mean ratio of PDE to ATP in unlocalized spectra acquired with TR 1 sec and in spectra localized to the basal ganglia (Table 3).

In the patients with overt hepatic encephalopathy, significant reductions were observed in the mean ratios of PME to ATP and PDE to ATP in both the unlocalized spectra, whether acquired with TR 1 sec or 5 sec, and the localized spectra (Table 3). The changes in the mean ratios of PME to ATP and PDE to ATP distinguished the patients with overt hepatic encephalopathy from those who were neuropsychiatrically unimpaired (Table 3). However, we noted no significant differences in mean
values for these metabolite ratios between the patients with subclinical hepatic encephalopathy and patients in the other two groups.

We found no significant correlations between these metabolite ratios and the patients' liver function parameters, Child grading, EEG mean cycle frequency, psychometric performance, blood ammonia concentrations or PSE sum.

We noted no consistent changes in the mean ratios of PCr to ATP or Pi to ATP in the total patient population (Table 2), and no changes in these metabolite ratios could be related to neuropsychiatric status (Table 3).

**DISCUSSION**

In our study of chronic hepatic encephalopathy, we found significant reductions in the ratios of PME to ATP and PDE to ATP in the cerebral $^{31}$P MR spectra from the total patient population compared with cerebral spectra from healthy volunteers. These reductions were correlated with the patients' neuropsychiatric status. It is possible that greater differences would have been observed between the patients with the subclinical and overt forms of the syndrome if all the patients had been studied in the untreated state or with more florid clinical signs. However, untreated patients are not often encountered, and when the condition is severe MRS examination may not be feasible.

Differences between the patient and reference populations were often greater in the unlocalized spectra than those localized to the basal ganglia. This may in part reflect the lower SNR in the localized spectra (Fig. 2). This in turn is a reflection of the smaller volume from which these spectra were obtained compared with the unlocalized spectra from the entire head. The time for data acquisition was determined by the small voxel size whether a single voxel or multivoxel technique was used. The use of a 4D-CSI technique allowed us to look at areas of the brain other than the basal ganglia, if necessary, without selecting a priori the region of interest, and did not prolong the examination time for this study.

Few cerebral $^{31}$P MR studies have been undertaken in patients with chronic liver disease, and there is little consensus in the published findings (3–7). This reflects both the small, rather heterogeneous patient populations studied and the variations in the MR localization techniques employed. Ross and coworkers (3) observed a significant decrease in intracerebral Pi, relative to PCr and ATP, in a group of eight cirrhotic patients with chronic hepatic encephalopathy; this they interpreted as indicating a defect in cerebral energy metabolism. Bar-
birolı and colleagues (7) observed markedly decreased PCr in four patients with overt hepatic encephalopathy, accompanied by increased Pi in three. These patients showed a significant increase in calculated ADP, a marked increase in relative velocity of ATP biosynthesis and a corresponding reduction in the phosphorylation potential. Similar but less striking reductions were observed in PCr in eight cirrhotic patients who were neurologically unimpaired, three of whom also showed increased Pi. The same group reported 28 patients in whom the ratio of ATP to ADP*Pi was reduced (5). These changes were interpreted as evidence of abnormal brain energy metabolism. Chamuleau and coworkers (4) observed no significant changes in cerebral 31P MR spectra acquired from 10 patients with cirrhosis and chronic hepatic encephalopathy and (in particular) observed no changes in intracerebral pH, PCr or ATP. Similarly, in this study we found no changes in the ratios of Pi to ATP and PCr to ATP.

The relationship between changes in cerebral energy metabolism and the pathogenesis of hepatic encephalopathy is unclear. Changes occur in cerebral energy metabolism in patients with severe hepatic encephalopathy; these appear as reductions in cerebral blood flow and in cerebral oxygen and glucose consumption (20-23). These changes reverse, after treatment, in parallel with improvements in the clinical status (20, 21, 23, 24). In animal models, however, changes in cerebral energy levels are delayed until after the onset of coma (25), suggesting that alterations in cerebral energy metabolism represent a secondary rather than a causal phenomenon in the genesis of the syndrome.

Our findings of reduced ratios of PME to ATP and PDE to ATP in the cerebral 31P MR spectra acquired from cirrhotic patients with subclinical or overt hepatic encephalopathy are therefore unique. The PME peak is multicomponent and includes contributions from phosphoethanolamine and phosphocholine, intermediates on the pathway of phospholipid membrane synthesis (26), as well as contributions from AMP and glycolytic intermediates (27). The PDE peak is also multicomponent and contains contributions from glycerophosphorylcholine and glycerophosphorylethanolamine, intermediates on the pathway of phospholipid membrane breakdown (26), as well as a contribution from endoplasmic reticulum. These compounds cannot be separated at the magnetic field strength (1.5 T) used in this study.

The reasons for the reductions that we observed in the PME and PDE signals in the brains of patients with hepatic encephalopathy remain unclear. However, reduced contributions of choline-containing compounds have been shown in the cerebral spectra of patients with hepatic encephalopathy with proton MRS (1H MRS) (28-30). Such changes in the choline resonance of 1H MR spectra may correlate with the observed reductions in

---

**Table 2. Comparison of metabolite ratios in the patient and reference populations**

<table>
<thead>
<tr>
<th>Metabolite ratio</th>
<th>Reference population (n = 10)</th>
<th>Patients (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlocalized spectra</td>
<td>Localized spectra</td>
</tr>
<tr>
<td>PME/ATP</td>
<td>TR 1 sec</td>
<td>TR 5 sec</td>
</tr>
<tr>
<td></td>
<td>1.16 ± 0.23*</td>
<td>1.29 ± 0.21</td>
</tr>
<tr>
<td>PDE/ATP</td>
<td>4.36 ± 1.17</td>
<td>4.09 ± 0.87</td>
</tr>
<tr>
<td>Pi/ATP</td>
<td>0.80 ± 0.10</td>
<td>0.90 ± 0.23</td>
</tr>
<tr>
<td>PCr/ATP</td>
<td>1.24 ± 0.45</td>
<td>1.67 ± 0.45</td>
</tr>
</tbody>
</table>

Localized spectra were acquired from 13 healthy volunteers and 23 cirrhotic patients.

*Data expressed as mean ± S.D.

Values significantly different between reference and patient populations:

-*p < 0.0005, "p < 0.0001, "p < 0.005, "p < 0.001, /p < 0.01, /p < 0.05.

---

**Table 3. Comparison of metabolite ratios in the patient subgroups, graded according to neuropsychiatrie status**

<table>
<thead>
<tr>
<th>Neuropsychiatric status</th>
<th>PME/ATP</th>
<th>PDE/ATP</th>
<th>Pi/ATP</th>
<th>PCr/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpaired (n = 6)</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
</tr>
<tr>
<td></td>
<td>1.12 ± 0.12</td>
<td>1.07 ± 0.33</td>
<td>3.69 ± 0.25</td>
<td>4.48 ± 1.54</td>
</tr>
<tr>
<td>Subclinical encephalopathy (n = 8)</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
</tr>
<tr>
<td></td>
<td>0.98 ± 0.16*</td>
<td>0.70 ± 0.43*</td>
<td>3.72 ± 0.75</td>
<td>3.09 ± 0.79*</td>
</tr>
<tr>
<td>Overt encephalopathy (n = 19)</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
<td>Unlocalized, TR 5 sec</td>
<td>Localized*</td>
</tr>
<tr>
<td></td>
<td>0.81 ± 0.20*</td>
<td>0.69 ± 0.31*</td>
<td>3.11 ± 0.62*</td>
<td>3.60 ± 1.55*</td>
</tr>
</tbody>
</table>

Localized spectra were acquired from 13 healthy volunteers and 23 patients.

Reference values, expressed as mean (range): 1.29 (1.03-1.90), 1.23 (0.63-1.95), 4.09 (2.38-5.55), 6.19 (3.44-9.30), 0.90 (0.63-1.43), 0.91 (0.47-1.67), 1.67 (0.7-2.38), 6.95 (0.59-1.60).

*Data expressed as mean ± S.D.

Values significantly different between reference and patient populations: /p < 0.005, /p < 0.05, /p < 0.0001, /p < 0.01.
PME and PDE found in the $^{31}$P MR spectra in this study because phosphocholine is a major component of the PME signal, and glycerophosphorylcholine is a major part of the PDE signal. This suggests that cerebral phospholipid metabolism is altered in these individuals. A reduced contribution of phosphocholine to the PME peak may not be sufficient to explain the observed reductions in the ratio of PME to ATP in this study, but this may also be explained in part by a reduced contribution of glycolytic intermediates to this peak resulting from decreased glucose utilization (20-23, 31-34). Further studies using $^{13}$C MRS and positron emission tomography may help clarify these findings.

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REFERENCES

Cirrhosis of the human liver: an in vitro $^{31}$P nuclear magnetic resonance study

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Cirrhosis of the human liver: an in vitro $^{31}$P nuclear magnetic resonance study

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Abstract

Human livers with histologically proven cirrhosis were assessed using in vitro $^{31}$P NMR spectroscopy. Spectra were compared with those from histologically normal livers and showed significant elevations in phosphoethanolamine (PE) and phosphocholine (PC) and significant reductions in glycerophosphorylethanolamine (GPE) and glycerophosphorylcholine (GPC). There were no significant differences in spectra from livers with compensated and decompensated cirrhosis. These results help to characterise the alterations in membrane metabolism in cirrhosis of the liver.

Keywords: NMR, $^{31}$P; Cirrhosis; Phospholipid; Human; Liver

1. Introduction

The human liver responds to injury in broadly the same way, irrespective of the original causal agent [1]. Persistent alcohol abuse, viruses such as hepatitis B and hepatitis C, genetic disorders including haemochromatosis, Wilson’s disease and $\alpha$-antitrypsin deficiency, cholestatic conditions such as primary biliary cirrhosis and primary sclerosing cholangitis, certain drugs and autoimmune diseases all may provoke a series of events that ultimately lead to cirrhosis or irreversible liver damage [2].

Cirrhosis of the liver is a diffuse process, characterised by the formation of fibrous tissue and regrowth of hepatocytes in an abnormal nodular pattern [3]. Current assessment methods of the functional state of liver injury in cirrhosis are not entirely satisfactory, usually depending on a severity index obtained from a collection of laboratory parameters and clinical findings [4–7].

Nuclear magnetic resonance (NMR) spectroscopy is a non-invasive technique, which can be used to provide localised biochemical information on hepatic metabolic processes in vivo. A typical $^{31}$P NMR spectrum of the human liver in vivo contains resonances which may be assigned to phosphomonoesters (PME), phosphodiesters (PDE), inorganic phosphate (Pi) and nucleotide triphosphates (NTP) [8–12].

The PME and PDE resonances in hepatic spectra are multicomponent and the constituents cannot as yet be completely resolved at the magnetic field strengths employed in human in vivo NMR studies, despite the use of proton-decoupling techniques [13]. The PME resonance includes contributions from cell membrane precursors [14] and glycolytic intermediates [15]. The PDE resonance is also composite, containing information from cell membrane breakdown products [14] and from endoplasmic reticulum [16].

Previous human in vivo NMR studies have reported on
the elevation in PME/ATP and the reduction in PDE/ATP with increasing functional severity of cirrhosis [17,18]. However, the underlying metabolic abnormalities responsible for these observations have not been fully investigated.

In vitro NMR techniques on human tissue extracts have been successfully used to study the metabolic changes responsible for the in vivo PME and PDE signals in hepatic tumours and normal liver [15,19]. Although Menon and colleagues [18] reported on in vitro NMR findings from a small number of livers from patients with chronic liver disease, no systematic approach has been applied to the characterisation of the cirrhotic liver.

Therefore, the aim of this study was to characterise the metabolic changes observed by in vivo hepatic 31P NMR in cirrhosis of the liver. The results are discussed in the context of previous in vivo hepatic 31P NMR findings.

2. Materials and methods

Standard percutaneous liver biopsies do not yield enough tissue for in vitro NMR studies, and therefore samples of cirrhotic liver were taken during surgery for orthotopic hepatic transplantation. Liver tissue was obtained from 25 patients with histologically proven cirrhosis. Ten patients (40%) had primary biliary cirrhosis, seven (28%) post-viral cirrhosis, six (24%) primary sclerosing cholangitis, one (4%) Wilson’s disease and one (4%) alcoholic cirrhosis.

The severity of liver dysfunction was assessed using the Pugh’s score [4], obtained from clinical and biochemical data, acquired on the day of liver transplantation. This is the standard scoring system, which is used clinically, grading liver injury from 5 (best function) to 15 (worst function), taken from information comprising serum bilirubin, plasma albumin levels, prothrombin time and the presence/severity of ascites and hepatic encephalopathy.

The 25 liver samples were categorised into two groups: functionally compensated cirrhosis with a Pugh’s score ≤ 7 (n = 10) and functionally decompensated cirrhosis with a Pugh’s score ≥ 8 (n = 15) (Table 1).

Permission for this study was obtained from the Ethics Committees of the Royal Postgraduate Medical School, London, and the Royal Free Hospital and School of Medicine, London. All patients provided written, informed consent.

2.1. Sample collection

Two investigators were present in the operating theatre to obtain tissue samples from each recipient liver. In every case, 6–8 representative sugar lump sized pieces of liver were freeze-clamped in liquid nitrogen with minimum possible ischaemic time (2–7 min). This was performed ex vivo within 3 min of hepatectomy in 22 cases. All samples were stored separately in a liquid nitrogen dewar until further processed.

### Table 1

| Tissue type | Serum bilirubin (µmol/l) | Plasma albumin (g/l) | Prothrombin time (s) | Pugh’s score *
|-------------|--------------------------|----------------------|----------------------|----------------
| Compensated | 177 (n = 10) 40 (35-60) | 14 (31-48) 7 (13-16) | 17 (6-7) 10 (8-12) |
| Decompensated | 143 (n = 15) 31 (22-40) | 17 (31-48) 7 (6-7) | 10 (8-12) |

Data are means (range values).


All information was obtained preoperatively, on the day of liver transplantation.

2.2. Reference data

Reference data were obtained from wedge biopsy samples of liver, taken from 6 patients undergoing laparotomy for surgical treatment of pancreatitis. In each case, contiguous samples of liver tissue were found to be histologically normal on examination [15].

2.3. Tissue extract preparation

The wet weight of each sample was between 560 mg and 2310 mg. Twelve per cent perchloric acid (PCA) was added to the still-frozen samples, in a ratio of 5 ml/g of liver tissue. Each sample was ground down under liquid nitrogen with a mortar and pestle and then allowed to thaw, before centrifugation at 3000 rpm for 10 min. The supernatant was separated, neutralized with 3 M KOH, freeze-dried and reconstituted in D2O. The pH was readjusted to 7.5, after the addition of 100 mmol/l of EDTA to chelate any paramagnetic metal ions present. Absolute quantification of metabolites was achieved by adding known amounts of methylene diphosphonate (MDP) and/or phosphocreatine (PCr) to the perchloric acid extracts. These acted as internal reference standards for chemical shift assignments of the resonances observed.

2.4. NMR methods

All NMR spectroscopy measurements were performed at room temperature. Proton-decoupled 31P NMR spectra were obtained using a high resolution NMR spectroscopy system (operating at 11.7T), from the perchloric acid extracts of liver tissue, with 16 K data points and a 45° pulse angle applied at intervals of 1 s. Corrections for T1 relaxation were made using samples run with a repetition time of 20 s. Metabolites were assigned using the methods
we have previously described [15]. The chemical shift of each metabolite was found and subsequently confirmed by the use of 'spiking' with known compounds [15].

2.5. Data processing

The free induction decay (FID) was zero filled to 32 K and Fourier transformed after line-broadening of 5 Hz. Peak areas for PE, PC, GPE, GPC, MDP and/or PCr were obtained, using the NMR |® spectral processing program (New Methods Research, E. Syracuse, USA) on a SUN SPARCstation 10 (Sun Microsystems, Mountain View, CA, USA). The data were fitted to Lorentzian functions.

Fig. 1. Typical proton-decoupled 31 P NMR spectrum of perchloric acid extract prepared from histologically normal liver tissue, (a) Full spectrum; (b) PME and PDE regions. Abbreviations: PME, phosphomonoesters; PDE, phosphodiesters; NAD, NADH + NAD; NTP, nucleotide triphosphates; NDP, nucleotide diphosphate; PE, phosphoethanolamine; PC, phosphocholine; GPE, glycerophosphorylethanolamine; GPC, glycerophosphorylcholine; PCr (phosphocreatine) and MDP (methylene diphosphonate) were added as internal reference standards.

Fig. 2. Typical proton decoupled 31 P NMR spectrum of perchloric acid extract from liver tissue with histologically proven cirrhosis, (a) Full spectrum, (b) PME and PDE regions. Abbreviations: PME, phosphomonoesters; PDE, phosphodiesters; NAD, NADH + NAD; NTP, nucleotide triphosphates; NDP, nucleotide diphosphate; PE, phosphoethanolamine; PC, phosphocholine; GPE, glycerophosphorylethanolamine; GPC, glycerophosphorylcholine; PCr (phosphocreatine) and MDP (methylene diphosphonate) were added as internal reference standards.

2.6. Statistical analysis

Since the data were not normally distributed, non-parametric statistical analysis was applied. Values for metabolite concentrations in the patient and reference populations were compared using the Mann-Whitney U-test. A P-value of < 0.05 was considered significant. All metabolite concentrations are quoted as mean values ± 1 standard deviation.

3. Results

A typical 31 P NMR spectrum from a PCA extract of normal liver contains resonances arising from PME, PDE,
Table 2
Concentrations of metabolites obtained from in vitro $^3$P NMR spectra from histologically normal and cirrhotic liver tissue

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Metabolite concentrations ($\mu$mol/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
</tr>
<tr>
<td>Normal liver (n = 6)</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>All cirrhosis (n = 25)</td>
<td>1.04 ± 0.75</td>
</tr>
<tr>
<td>Compensated cirrhosis (n = 10)</td>
<td>1.28 ± 0.70 a</td>
</tr>
<tr>
<td>Decompensated cirrhosis (n = 15)</td>
<td>0.88 ± 0.76 d</td>
</tr>
</tbody>
</table>

Data are mean values ± 1 S.D.
Significant difference from the reference population: a $P < 0.0005$, b $P < 0.05$, c $P < 0.0001$, d $P < 0.001$, e $P < 0.01$.

NTP, NDP and Pi (Fig. 1). The PME region of the spectrum consists of over 10 resonances, including signal from PE, PC, AMP, 2,3-DPG, coenzyme A, glucose 6-phosphate, glyceral 1-phosphate, 3-phosphoglycerate and ribose 5-phosphate [15–19]. The PDE region contains two major resonances, GPE and GPC [15,19–21].

Most of these resonances vary markedly with ischaemia and it was therefore only sensible to quantify the more stable compounds, namely PE and PC from the PME region and GPE and GPC from the PDE region of the spectrum [22–24].

The signal intensity of the PE and PC resonances was increased and the GPE and GPC resonances reduced in spectra from liver with histologically proven cirrhosis (Fig. 2) when compared to spectra from histologically normal liver. The metabolite concentrations ($\mu$mol/g wet weight of liver tissue) are summarised in Table 2.

All cirrhotic livers showed significantly higher PE (1.04 ± 0.75 vs 0.16 ± 0.03; $P < 0.0005$) and PC concentrations (0.41 ± 0.37 vs 0.16 ± 0.04; $P < 0.05$) and significantly lower GPE (0.29 ± 0.37 vs 2.35 ± 0.46; $P < 0.005$) and GPC concentrations (0.14 ± 0.26 vs 2.46 ± 0.37; $P < 0.0001$) than normal tissue (Table 2).

There was no significant difference between PE, PC, GPE and GPC concentrations from livers with functionally compensated cirrhosis and those from livers from functionally decompensated cirrhosis (Table 2).

There were regional variations in metabolite concentrations when liver samples from different areas of the same liver were analysed. Table 3 illustrates these variations in metabolite levels in a patient with compensated cirrhosis.

There was no correlation between individual biochemical indices (serum bilirubin, plasma albumin and prothrombin time) or clinical parameters of liver dysfunction (presence of ascites and hepatic encephalopathy), measured on the day of the transplant operation, and PE, PC, GPE and GPC concentrations from the liver extracts.

### Table 3

<table>
<thead>
<tr>
<th>In vitro $^3$P NMR: Variations in metabolite concentrations obtained from different regions of the same liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolite concentration</td>
</tr>
<tr>
<td>PE (0.09–0.24) *</td>
</tr>
<tr>
<td>PC (0.11–0.23) *</td>
</tr>
<tr>
<td>GPE (1.79–2.71) *</td>
</tr>
<tr>
<td>GPC (2.09–2.83) *</td>
</tr>
</tbody>
</table>

All values expressed as $\mu$mol/g wet weight of liver tissue.
* Reference range.

4. Discussion

This study used in vitro $^3$P NMR to describe the changes in aqueous soluble membrane components in livers with histologically proven cirrhosis, compared to normal human liver tissue.

Several human in vivo $^3$P NMR studies of the liver have shown abnormalities in PME, PME/ATP, PME/PDE and PDE/ATP in patients with cirrhosis [17,18,25–27]. Two of these studies have correlated the functional severity of liver injury in cirrhosis with an elevation in PME/ATP and a reduction in PDE/ATP [17,18].

Our study attempted to investigate the underlying metabolic changes responsible for these in vivo spectral appearances in man. Unfortunately, a limitation of human tissue characterisation by in vitro methods is the unavoidable period of ischaemia during biopsy collection. Only quantification of PE, PC, GPE and GPC was attempted, as the other metabolites that comprise the PME and PDE peaks are known to alter radically from the in vivo situation during periods of ischaemia [15,19]. Hachisuka and colleagues [28] noted that in rat liver subjected to prolonged periods of ischaemia beyond 30 min, PC and PE were relatively stable, while GPE and GPC decreased. However, post-mortem studies of human brain and animal liver have indicated that the levels of PE, PC, GPE and GPC are not significantly affected by periods of ischaemia of up to one hour [22–24]. In our study much shorter periods of ischaemia were encountered. Twenty-two of the 25 tissue samples from cirrhotic liver were collected within 3 min of hepatectomy, while in the three tissue samples the ischaemic period was up to 7 min.

Comparison of the $^3$P NMR spectra of PCA extracts from cirrhotic liver and histologically normal tissue showed increased concentrations of PE and PC and decreased concentrations of GPE and GPC from the diseased tissue.
Regional variations in metabolite concentrations were observed from samples obtained from different areas of each individual liver.

Our results suggest that increased concentrations of PE and PC may be responsible for elevation in PME/ATP observed in vivo [17,18,27]. Similarly, the reduction of PDE/ATP seen in vivo [17,18] may be explained, at least in part by the reduction in GPE and GPC which we have noted. Endoplasmic reticulum is also an important component of the PDE resonance in vivo [16,29], but its relative contribution in the human cirrhotic liver is unclear and requires further study.

The predominant contribution of PC and PE are as intermediates on the pathway of phospholipid biosynthesis [14]. GPE and GPC are phospholipid breakdown products [14]. Increased PE [30–33] and PC [34] have been observed in the regenerating rat liver and in other conditions of rapid cellular proliferation, such as in hepatic tumours [15,19,35]. Lymphomatous infiltration of the liver is also associated with elevated PE levels [35].

The hallmark of cirrhosis is abnormal regrowth of liver tissue in a nodular pattern. This occurs in the presence of increased fibroblastic activity [3]. The increase in PE and PC in our study may therefore be due to increased cell turnover as the cirrhotic liver attempts to regenerate. Either hepatocyte regeneration or the laying down of fibrous tissue, during the cirrhotic process, may be responsible for this phenomenon.

GPE and GPC levels are reduced in rapidly proliferating cells [15,19,32,33] and, in conditions of increased cell turnover such as the failing cirrhotic liver, it may be reasonable to expect reduced levels of these cell membrane degradation products.

Unlike the in vivo studies where there was an elevation in PME/ATP and PDE/ATP, correlated with the functional severity of liver injury [17,18], there was no statistical difference between metabolite levels from functionally compensated and decompensated cirrhotic liver in our study. This may partially reflect the arbitrary nature of the clinical grading system [4], which is subject to a number of extrahepatic influences. Furthermore, the regional variation in metabolites concentrations that we observed within each individual liver highlights the fact that cirrhosis is not a uniform process. Therefore, the lack of distinction between liver samples from patients with compensated and decompensated cirrhosis may also be a reflection of the varying composition of these tissue samples.

Further studies correlating in vivo $^{31}$P NMR spectral abnormalities with in vitro $^{31}$P NMR appearances and electron microscopy of liver tissue to assess the NMR contribution of endoplasmic reticulum are required. However, the results of this study suggest that the changes in PE, PC, GPE and GPC are responsible, to a large extent, for the PME/ATP and PDE/ATP abnormalities seen in patients with cirrhosis of the liver.

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