Magnetic Resonance Spectroscopy: Principles and Techniques: Lessons for Clinicians

Joshua M. Tognarelli^{*}, Mahvish Dawood^{*}, Mohamed I.F. Shariff^{*}, Vijay P.B. Grover^{*}, Mary M.E. Crossey^{*},
I. Jane Cox[†], Simon D. Taylor-Robinson^{*}, Mark J.W. McPhail^{*}

^{*}Liver Unit, Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Imperial College London, London, United Kingdom and

[†]The Foundation for Liver Research, Institute of Hepatology, 69-75 Chenies Mews, London WC1E 6HX, United Kingdom

Magnetic resonance spectroscopy (MRS) provides a non-invasive ‘window’ on biochemical processes within the body. Its use is no longer restricted to the field of research, with applications in clinical practice increasingly common. MRS can be conducted at high magnetic field strengths (typically 11–14 T) on body fluids, cell extracts and tissue samples, with new developments in whole-body magnetic resonance imaging (MRI) allowing clinical MRS at the end of a standard MRI examination, obtaining functional information in addition to anatomical information. We discuss the background physics the busy clinician needs to know before considering using the technique as an investigative tool. Some potential applications of hepatic and cerebral MRS in chronic liver disease are also discussed. (J CLIN EXP HEPATOL 2015;5:320–328)

The biomedical applications of nuclear magnetic resonance (NMR) are twofold: magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). The applications of MRS as a research tool are extremely diverse, encompassing studies on isolated cells, body fluids and perfused organs at high magnetic field strengths in an experimental, laboratory-based setting and also *in vivo* studies using clinical MR systems.¹ *In vivo* clinical MRS on whole-body MRI scanners has been used to study the metabolism of well-defined regions of the human body, affording a non-invasive ‘metabolic window’ on a wide range of biochemical processes in the body, including the composition and function of human organs *in vivo*.² Clinical MRS developments have exploited many of the advances in MRI at the magnetic field strengths now used (typically 1.5–3.0 T) and the use of magnetic field

gradients. The sensitivity and spatial resolution of MRS is a limiting factor *in vivo*, but parallel utilisation of *in vitro* MR spectroscopy of tissue extracts, body fluids and cell lines at much higher magnetic field strengths (typically 11.7–14.1 T) allows more definitive interpretation of the *in vivo* data. In this article, we aim to equip the clinician with knowledge of the background physics involved in MRS, so that informed decisions can be made for research studies.

NUCLEAR MRS

NMR refers to the behaviour of atoms subjected to a magnetic field. The phenomenon was first described in 1946 by Bloch and Purcell. Atoms with an odd mass number such as ¹H, ³¹P and ¹³C possess the quantum property of “spin” and behave as dipoles aligning along the axis of an applied magnetic field (Figure 1). During relaxation following excitation, radiofrequency signals are generated which can be expressed as a frequency spectrum. Hydrogen is the most abundant atom in living organisms and using high power magnetic fields on *in vitro* samples, high resolution metabolic spectra can be obtained with clearly defined metabolite peaks of small molecules (<2 kDa).

NUCLEAR SPIN AND ORIENTATIONS

Nuclear resonance occurs because the nuclei of at least one of the isotopes of most elements possess a magnetic moment. A magnetic moment arises because the nucleus may have ‘spin’, and is also charged. “Spin” can be understood as the nucleus of an atom spinning around its own axis.³

When placed in a constant magnetic field, nuclei that possess spin can be excited, the energy of the magnetic moment depends on the orientation of the nucleus with respect to that field.³ Application of electromagnetic

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Address for correspondence: Joshua Tognarelli, Liver Unit, Department of Medicine, 10th Floor QEOM Wing, St Mary’s Hospital, Imperial College London, Praed Street, London W2 1NY, United Kingdom.

Tel.: +44 207 886 6454; **fax:** +44 207 402 2796.

E-mail: jt2110@imperial.ac.uk

Abbreviations: CPMG: Carr-Purcell-Meiboom-Gill sequence; CSI: chemical shift imaging; FID: free induction decay; K: Kelvin; KEGG: Kyoto Encyclopedia for Genes and Genomes; MR: magnetic resonance; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; MSEA: metabolite set enrichment analysis; NMR: nuclear magnetic resonance; NOESY: nuclear Overhauser enhancement spectroscopy; PC: principal components; PCA: principal components analysis; PLS-DA: partial least squared discriminant analysis; PRESS: point-resolved spectroscopy; STEAM: stimulated echo acquisition mode; T: Tesla; T₁: spin-lattice relaxation; T₂: spin-spin relaxation; TE: echo time; TMAO: trimethylamine N-oxide; TR: repetition time

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Figure 1 Precession of protons aligned to a magnetic field (B_0).

radiation at a suitable frequency can stimulate transitions between high and low energy states, this transition in energy level providing the basis for NMR spectroscopy, as the energy absorbed can be detected.^{3,4}

In an applied magnetic field, the magnetic moments of nuclei become oriented relative to the direction of the applied field in a number of ways, determined by the nuclear spin quantum number, I . For protons, $I = 1/2$, so the number of orientations of proton magnetic moments in the applied field is $(2 \times 1/2 + 1) = 2$, i.e. parallel and anti-parallel to the applied field direction.⁵ These two orientations are characterised by two different energy levels (the parallel orientation being lower in energy than the anti-parallel) whose separation ΔE depends on the applied field strength, B_0 , as given in equation, where h is Planck's constant (Figure 2).⁴

Electromagnetic radiation in the radio-frequency range causes transitions between the two energy levels, giving the possibility of ^1H NMR spectra. Since $\Delta E = h\nu$, it follows that the resonance frequency ν is proportional to the field strength B_0 .

$$\nu = \frac{\gamma}{2\pi} \times B_0$$

Resonant absorption by nuclear spin will occur only when electromagnetic radiation of the correct frequency (Larmor precession rate) is applied to match the energy difference between the

Figure 2 Energy levels depicted on a $1/2$ I spin nuclei.

nuclear spin levels in a constant magnetic field of appropriate strength.

CHEMICAL SHIFT

In a molecule, the magnetic field that a particular proton experiences is influenced by that due to the motions of nearby electrons (the chemical environment to which it is subjected). Differently sited protons experience slightly different effective applied fields and resonate at slightly different frequencies; it is this which gives ^1H NMR its diagnostic value, as this property can be used to discern different proton environments within molecules.⁴ This effect is called 'chemical shift' and if a nucleus in a specific chemical group is shielded to a higher degree by a higher electron density of its surrounding molecular orbital, then the NMR frequency will be shifted "up field" (that is a lower chemical shift), whereas if it is less shielded by surrounding electron density, then the NMR frequency will be shifted "downfield" (that is, a higher chemical shift). Thus, the magnetic environment experienced by each MR sensitive nucleus may be different. Although all nuclei are dominated by the static magnetic field strength, B_0 , and by the applied field B_1 , they will also experience a local magnetic field due to the magnetic fields of the electrons within their immediate chemical environment. Thus, the degree of shielding or enhancement of the local magnetic field by electron currents depends upon the exact electronic environment, which is a function of the precise chemical structure of the molecule.⁴

SPIN-SPIN COUPLING

When protons occur in more than one kind of environment within a molecule, circumstances may allow their spins to interact with one another. The influence of one proton's spin on another is due to the shielding effects of its electrons, which can cause the magnetic energy experienced by a neighbouring proton to be slightly stronger or weaker if the magnetic moment of the neighbouring proton is parallel or perpendicular to the magnetic force

applied. In reality, roughly half of the neighbouring protons are found to be parallel and half perpendicular to the external magnetic field. Therefore, a proton's NMR signal can be found as a split peak, with one peak shifted downfield slightly on NMR and one shifted upfield slightly. This effect is known as spin-spin coupling and it can be seen in different forms in MRS. Its interpretation can allow for finely detailed analysis of the molecular structure being analysed.⁴

SIGNAL PRODUCTION AND OBTAINING A SPECTRUM

The rate of signal decay is characterised by both spin lattice (T_1) and spin-spin (T_2) relaxation times. Resonance is obtained by superimposing weak oscillating field B_1 on the field B_0 generated by a receiver coil⁵; signal production is achieved by applying a short, intense pulse of radio-frequency energy to the sample under test, which is absorbed by the nuclei present. After the applied pulse has terminated, the sample emits signals for a time, while various transitions return to their equilibrium state.³ The resulting signal, called free induction decay (FID), consists of a superimposition of all the resonance from the sample, which have to be decoded in order to obtain the familiar presentation in the form of a spectrum.³ The decoding process is done by an arithmetical process known as *Fourier transformation* (Figure 3).³

The phenomenon of chemical shift therefore gives rise to a MR frequency spectrum consisting of nuclei which resonate at different frequencies. The frequency depends on the exact magnetic field strength, so it is usually expressed in dimensionless units (parts per million, ppm), by reference to a specific material; in ^1H MR spectroscopy, this is often water at 4.7 ppm. ^1H and ^{31}P are the main nuclei investigated in *in vivo* clinical MRS, but ^{13}C , ^{23}Na and ^{19}F are also amenable to MRS investigation, if the right equipment exists. Peaks in the MR spectra are also called resonances. Some metabolites may be split into two (doublet) or more sub-peaks. The area beneath the peak represents the concentration of the metabolite. Absolute quantification of metabolites is theoretically possible, but can be difficult to achieve accurately owing to factors including T_1 and T_2 effects.⁶ Thus, for *in vivo* MRS, results

are usually reported as metabolite ratios to a stable metabolite occurring naturally in tissue, such as creatine. A number of other techniques can also be employed to increase result reliability.

MRS IN PRACTICE

Protons will resonate in the form of a sine wave and each of these waves will possess a phase. Not all the protons in the samples will possess the same phase. Therefore, when the FID is Fourier transformed, some resonances will display positive peaks, some will display negative peaks and some in between. By multiplying the spectrum by a phase correction, which can be applied to the whole spectrum, all peaks can be corrected to positive and symmetrical line shapes.

BASELINE CORRECTION

Baseline errors can be corrected using automated or manual software so that all spectra have a baseline starting at a y-value of 0.

EXPONENTIAL WEIGHTING OR LINE-BROADENING FUNCTIONS

To reduce the contribution of background noise in the spectrum, the FID can be multiplied by a weighting or "line-broadening" function. This has the effect of truncating the FID tail, so that resonances from noise are not Fourier transformed, resulting in an increased signal-to-noise ratio.

ZERO-FILLING

The NMR signal is collected as a series of data points by the receiver coil. By adding an equal number of zeros to the original number of data points (done simply by the computer), the number of data points increases, which in turn improves line shapes in the resultant NMR spectrum. This manipulation has no effect on signal resolution, and it is purely cosmetic.

NMR SYSTEMS

Magnetic fields of the strength used for *in vitro* high resolution NMR spectroscopy can only be generated by superconducting magnets. The field is generated by passing a current through a wire coil. The wire is super-cooled to a temperature of less than 6 K and is composed of filaments of one metal or alloy embedded in another (solenoid: typically tin, copper and niobium) which allows its conductive resistance to be reduced to almost 0, thereby allowing high currents to flow and perpetually persist. The

Figure 3 Fourier transformation from free induction decay to MR spectrum. Key: FID, free induction decay; FT, Fourier transformation.

wire coil is immersed in liquid helium which in turn is surrounded by a bath of liquid nitrogen (77 K) to prevent the helium from evaporating. Passing vertically through the centre of the machine is a room temperature zone into which the sample is placed (Figure 4).

SHIMMING

To obtain precision, the magnetic field needs to be as homogenous across the sample as possible. The magnet alone, even though it generates a very strong field, is not able to achieve this. Shim coils are small wire coils placed strategically around the sample, through which current is transmitted, generating small, local magnetic fields which can be individually altered to provide a near homogenous field.

TUNING AND LOCKING

“Locking” the field can also be performed to compensate for small, undesired fluctuations in field homogeneity. This is achieved by continuous monitoring of a designated signal (usually deuterated hydrogen) and adjusting the field by small amounts to keep this signal at the same frequency.

DATA ACQUISITION

In vivo, single voxel spectroscopy or chemical shift imaging are most commonly used for MRS. Single voxel spectroscopy defines voxels of interest within an organ using gradients. Voxel size used is predefined by the user. Use of smaller voxels requires a higher number of signal

averages to be acquired in order to improve the signal-to-noise ratio, and this leads to a longer scan duration. Chemical shift imaging (CSI) uses a matrix of voxels to form the spectra. Theoretically, this can be done in all three directions, but practically, is usually done in one plane, giving 2D single slice CSI. Single voxel spectroscopy creates superior image quality, but CSI allows greater anatomical coverage.

SINGLE VOXEL SPECTROSCOPY

MRS peak amplitude depends upon the spectroscopy sequence used, repetition time (TR) and echo time (TE). Ideally, when using a clinical whole-body magnet system, signal loss due to T_1 relaxation and T_2 decay should be avoided. This is best achieved with a TR of at least 2000 ms and a TE as short as possible, often 30–35 ms. A longer TE would attenuate the signal from unwanted macromolecule resonances, such as from lipids.⁷

IN VIVO MRS PULSE SEQUENCES

Numerous different *in vivo* MRS sequences have been developed for clinical whole-body MRS, differing in pulse sequences and localisation methods. Stimulated echo acquisition mode (STEAM) and point-resolved spectroscopy (PRESS) are commonly used.^{8,9} The signal intensity acquired with PRESS is twice as high as STEAM. Both can now be produced with short echo times.¹⁰ The major difference between PRESS and STEAM is how the signal is produced. STEAM uses magnetisation to form a stimulated echo, whereas PRESS measures spin echo.

Figure 4 Schematic diagram of a nuclear magnetic resonance system. Key: A, outer shell; B, liquid nitrogen; C, liquid helium; D, solenoid wire coil; E, shim coils; F, receiver and radiofrequency pulse coils; G, biofluid sample.

IN VITRO MRS PULSE SEQUENCES

At high magnetic field strengths, the characteristics of the molecular surrounding of the proton can also be manipulated by using different RF pulse sequences. For example, a nuclear Overhauser enhancement spectroscopy (NOESY) sequence uses three sequential 90° pulses to allow the detection of higher and lower molecular weight samples, while that of the Carr-Purcell-Meiboom-Gill sequence (one 90° pulse and a repetitive loop of 180° pulses) suppresses larger weight molecular resonances and accentuates lower weight species.^{11,12}

IN VIVO DATA ANALYSIS

When interpreting *in vivo* MRS data, a number of experimental factors contributing data accuracy must be considered: the actual hardware used (coil characteristics, receiver linearity, homogeneity of the field and the voxel), water suppression efficiency and localisation of voxel, pulse sequence and the analysis technique method used to quantify the data and the physical characteristics of the tissues.¹³ As water concentration differs between tissues in the body, calculations of metabolite concentrations using water as an internal reference can be affected if the tissue composition of a voxel is not able to be accurately determined.¹⁴ Another issue to consider is the numerous different software programs for MRS data analysis; analysis by different investigators can give varying results for identical spectra.¹⁵ Thus, when spectra are analysed by different investigators, there may be variability in the metabolite ratios.¹³

IN VIVO CEREBRAL PROTON SPECTROSCOPY-VISIBLE METABOLITES

While high resolution MRS obtained on body fluids at high magnetic field strengths will reveal a whole array of metabolites, the most important visible peaks on the cerebral proton MR spectrum *in vivo* at field strengths of 1.5–3.0 T are N-acetyl aspartate (NAA), choline (cho), creatine (Cr) myo-inositol (mI), the combined glutamine and glutamate peak (Glx) and lactate (Lac). At a TE of 30 ms, the NAA resonance is at 2.0 parts per million (ppm), cho at 3.2 ppm, Cr at 3.0 ppm, mI at 3.6 ppm, the Glx complex between 2.1 and 2.5 ppm and the lactate doublet around 1.3 ppm.

METABOLITE QUANTIFICATION IN IN VIVO MRS

Using *in vivo* MRS, metabolite concentrations are expressed as absolute values or ratios. Absolute quantification requires the use of reference solutions (known as “phantoms”) or

more often, using tissue water as a reference for the MR scanner.¹⁶ Absolute quantification is theoretically ideal, as it allows description of individual metabolite concentrations and variation in disease states. There are some methodological problems with absolute quantification however. If external reference solutions are used there is obvious concern regarding B_1 field inhomogeneity in the regions of interest. If water is used as an internal reference, then water content must be assumed, though this may differ in disease states. Additionally, different tissue classes may have different water contents: for example grey and white matter in the brain.¹⁶ Water forms the vast majority of brain tissue mass, present at over 10,000 times the concentration of most other metabolites (10 mmol/L); therefore, small errors in the assignment of the absolute water concentration can easily affect calculated relative concentrations of metabolites.¹⁴ Absolute quantification prolongs scanning time as T_1 and T_2 relaxation effects upon water and the metabolites needed to be accounted for, requiring additional measurements. Metabolite ratios assume that creatine concentration is stable within tissues during health and disease; this may be incorrect of course. However, this assumption is made at the same time and within the same region most often, so the error should be dynamic and therefore minimised.

IN VIVO MRS ANALYSIS

Integration or line-fitting the signal, after Fourier transformation, is the most common way to calculate the metabolite peaks, using proprietary software. Accuracy can be elevated through the use of a prior-knowledge based system, using previously obtained information regarding the characteristics of the spectrum, which are often different between differing hardware and acquisition sequences.¹⁷ JMRUI is an example of a prior-knowledge based software package, and it uses the AMARES algorithm.^{17,18} The use of prior knowledge also reduces user-dependent input, and therefore operator-dependent variability. Finally, analysis of spectroscopy data in the time domain, as JMRUI does, rather than the frequency domain is advantageous to reduce the impact of artefacts and unwanted noise within the data.¹⁵

IN VITRO METABOLIC PROFILING

Metabolic profiling is a general term encompassing “metabonomics”, which is the study of global metabolic responses to physiological, drug and disease stimuli and “metabolomics”, which aims to characterise and quantify all the small molecules in biofluid sample.^{19,20}

The most commonly used methods of metabolite characterisation are *in vitro* MRS and mass spectrometry (MS). The techniques are complimentary and each has advantages and disadvantages (Table 1). Sensitivity of MS is

Table 1 Comparison of Nuclear Magnetic Resonance and Mass Spectrometry.

Variable	NMR	MS
Sensitivity	Lower than MS (nanomolar)	Higher than NMR (picomolar)
Sample degradation	No	Yes
Reproducibility across labs	High	Moderate
Metabolite identification	Well categorised	Labour intensive

high, in some forms of gas chromatography reaching femtomolar levels, but samples are degraded during the run and metabolite identification can be challenging.^{21,22} Nuclear MRS displays lower sensitivity (nano- to millimolar) but samples remain intact and NMR spectral profiles have been extensively categorised.^{23–25}

Comprehensive metabolic profiles have been generated from biofluids including urine, serum, bile and intact tissue.^{24,26–32}

MULTIVARIATE STATISTICAL ANALYSIS FOR IN VITRO MRS

The data generated by multi-sample NMR spectroscopy can generate 30,000 variables (Figure 5). Much of these data, however, are collinear. As analyses are often untargeted, in that it is not known what differences may be present between groups, an alternative method of statistical comparison is required. Multivariate analysis allows the important differences between groups of data to be rapidly visualised reducing multidimensional data to two or three variables. Furthermore, the differences between patient groups may be characterised by a group of metabolite ratios rather than a single metabolite.

Multivariate statistical analysis in the form of principal components analysis (PCA) and partial least squared discriminant analysis (PLS-DA) are common tools used for this purpose.³³

Figure 5 Multiple variables are generated from metabolic profiling studies.

PCA AND OUTLIER EXCLUSION

PCA is an unsupervised analytical tool that provides an overview of complex data through an examination of the covariance structure, highlighting sample outliers and clustering. This is done by converting each sample into a coordinate in n -dimensional space, based on its metabolic profile and calculating the factors (which are combinations of these vector co-ordinates) which contribute to the greatest variation across a group of samples. Orthogonal factors, or principal components (PC), are then plotted against each other. Essentially, new variables (principal components) are created, based on the variance between metabolite profiles between samples and the samples plotted as coordinates with the new variables acting as x, y and occasionally, z axes. In this manner, samples which are similar cluster and those that are different spread apart.³⁴ A measure of distribution, akin to Gaussian distribution, can be overlaid to identify those samples that are outside of the 95% confidence level of distribution (the Hotelling's or Mahalanobis' distance). These samples are classed as outliers and it is likely that they contain an abnormally high or low level of a particular metabolite making them unrepresentative of the group. Furthermore, because PCA is a measure of variation, these samples are likely to influence multivariate analysis, heavily weighting principal components. It is therefore reasonable to exclude these samples on the basis of unfair bias.

PLS-DA

PLS-DA is a supervised analytical method which relates metabolite data to class membership, elucidating separation between the groups. As with PCA, each sample is placed in n -dimensional space based on its metabolic profile, but instead of variation between samples, variation between groups is modelled allowing the elucidation of variable differences between groups. Furthermore, models can be created to test the accuracy of this multivariate model in predicting the origin of a new sample.³⁵ These statistical techniques can be applied to data matrices generated from *in vitro* ^1H MRS, producing matrices with hundreds to thousands of variables. The multivariate analyses allow the highlighting of two or three of these variables (principal or partial least squared components).

CONFOUNDING FACTORS INFLUENCING THE ^1H NMR SPECTRUM IN VITRO

Consideration must be taken into account when analysing an *in vitro* spectrum as the human metabolome is influenced by a number of phenotypic, physiological and external factors. These include gender, age, BMI, diet, stress, medications, smoking, exercise, fasting and consumption of alcohol 24 h prior to collection.^{36,37} Attempts to

minimise the impact of such confounding factors include questionnaires involving a 24 h dietary history, comprising data on current medication use, alcohol consumption and lifestyle factors.³⁸ Knowledge of such factors can help in data analysis, though many of these factors are often poorly characterised.³⁶ Some studies have specifically identified confounding factors that influence the ¹H NMR spectrum, a few of which are emphasised include trimethylamine N-oxide (TMAO) production, which is highly influenced by diet. TMAO is higher in meat eaters than vegetarians and higher in fish eaters than meat eaters³⁹; the consumption of meat can significantly increase the metabolite 1-methylhistidine³⁶; mannitol is a sugar alcohol in urine that can be explained by the consumption of foods such as apples, pineapples, asparagus and carrots; increased dietary intake of creatinine or a protein-rich diet can increase daily creatinine excretion; urinary samples from East Asian and Western populations has significantly different metabolite excretion patterns.^{36,39} Additionally, males have a higher skeletal mass than females; thus different urinary levels of creatinine may be present in women samples.³⁶

IN VITRO ¹H MRS ADVANTAGES AND LIMITATIONS

The major advantages of *in vitro* ¹H MRS are that it measures metabolites directly and allows clear distinction between compounds. Additional benefits include robustness and reproducibility.³⁹ Furthermore, the method is rapid, non-destructive, uses minimal sample volumes, and requires limited sample preparation.³⁷ These qualities make *in vitro* MRS a reliable technique for identification of structures in biofluids, intact biopsy specimens, and tissues with minimal sample preparation.⁴⁰

The major disadvantages of *in vitro* MRS include a failure to detect low abundance metabolites, particularly those <5 μ M (e.g. nucleotides or neurotransmitters).³⁷ Moreover, some metabolites can be effectively “hidden” in spectra if they are co-resonant with higher concentration metabolites.³⁹ Furthermore, data analysis can be very complex and requires expert interpretation when identifying potential metabolites of importance. Metabolite set enrichment analysis (MSEA) and Kyoto Encyclopedia for Genes and Genomes (KEGG) are bioinformatics tool that can help interpret metabolic profiles into biologically meaningful contexts, by identifying key metabolites concentrations from pathways, genetic traits and phenotypic consequence, and this is particularly useful as a vast array of pathways related to phenotype have been identified.⁴⁰

APPLICATIONS

In recent years, there have been numerous metabonomic studies investigating biofluids in a variety of hepatic

conditions. For example, one area of great interest has been liver cancer. Shariff and colleagues, in two ¹H NMR spectroscopy studies, have investigated the urine of Nigerian and Egyptian populations, comparing urine samples from patients with cirrhosis and those with hepatocellular carcinoma.^{41,42} Multivariate analysis uncovered a clear distinction in urinary metabolites between the two cohorts. These included reduced urinary creatinine, glycine, hippurate, trimethylamine-N-oxide, and citrate while urinary carnitine, creatine and acetone were raised. It was hypothesised that changes in these metabolites in HCC provide insight into increased mitochondrial respiration, altered lipid metabolism and deranged chromosomal methylation patterns. These findings were replicated in a larger study from Gambia, Senegal and Nigeria (the PROLIFICA Study).⁴³

A similar study was conducted to profile serum samples metabolically from patients diagnosed with hepatocellular carcinoma (HCC).⁴⁴ Metabolites of interest were focused on impaired lipid metabolism; serum lipid concentration was reduced but acetate, the end product of lipid metabolism, was raised, suggesting accelerated lipid degradation.⁴⁴

Other areas of interest at high magnetic field strength have been in biomarker development for cholangiocarcinoma using bile collected at ERCP, non-invasive serum biomarkers of fibrosis development in chronic hepatitis C and prediction of outcome following an episode of acute liver failure using serum and urine.^{45–47} All these studies were able to delineate disease-specific metabolic profiles which were clearly different from healthy and/or disease controls. The *in vitro* MRS technique on body fluid samples has a role both in large-scale population-based screening without specific *a priori* hypotheses and in demonstrating the response of the individual to therapy.⁴⁸

At clinical magnetic field strengths, whole body *in vivo* MRS has provided insight into pathogenic mechanisms of cell turnover in cirrhosis,^{49–51} the process of allograft rejection following liver transplantation,⁵² changes in cognitive function, osmolyte concentrations and low-grade cerebral oedema in hepatic encephalopathy and the mechanisms underlying fatigue in primary biliary cirrhosis.^{53,54}

FIVE YEAR VIEW

Liver disease is ripe for investigation by metabolic profiling techniques and a large number of research articles will be devoted to these clinical problems in the future. While *in vivo* MRS sequences can easily be added to standard clinical MRI examinations if the right software exists on the clinical scanner, we would expect that a combination of high-field *in vitro* MRS and mass spectroscopy techniques will be employed for future biofluid studies, as the potential limitations of *in vitro* MRS in terms of detection and identification of low concentration metabolites is a pertinent issue. The challenge for clinicians involved in this area

is the validation of results gleaned from hitherto small studies and the leap to translation into clinical practice. Close collaboration between scientists, statisticians and hepatologists is required to allow these techniques to be tested against present 'gold standards' so that the promise of non-invasive diagnostic and monitoring metabonomics tests can be realised.

CONFLICTS OF INTEREST

The authors have none to declare.

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