



## Review

# Quantitative *in vivo* neurochemical profiling in humans: where are we now?

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## Abstract

Proton nuclear magnetic resonance spectroscopy of biofluids has become one of the key techniques for metabolic profiling and phenotyping. This technique has been widely used in a number of epidemiological studies and in a variety of health disorders. However, its utilization in brain disorders is limited due to the blood–brain barrier, which not only protects the brain from unwanted substances in the blood, but also substantially limits the potential of finding biomarkers for neurological disorders in serum. This review article focuses on the potential of localized *in vivo* proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) for non-invasive neurochemical profiling in the human brain. First, methodological aspects of <sup>1</sup>H-MRS (data acquisition, processing and metabolite quantification) that are essential for reliable non-invasive neurochemical profiling are described. Second, the power of <sup>1</sup>H-MRS-based neurochemical profiling is demonstrated using some examples of its application in neuroscience and neurology. Finally, the authors present their vision and propose necessary steps to establish <sup>1</sup>H-MRS as a method suitable for large-scale neurochemical profiling in epidemiological research.

**Key words:** <sup>1</sup>H-MRS, methodology, spectral quality, neurochemical profiling, CNS disorders

## Key Messages

- *In vivo* neurochemical profiling in the human brain is feasible, but the widespread use requires the implementation of the most advanced methodologies of <sup>1</sup>H-MRS.
- There is a widening gap between what is feasible at specialized MR research centres and what is currently available for routine clinical practice.
- The software upgrade ( $B_0$  shimming, localization pulses sequence) on clinical 3T MR scanners is sufficient for a significant improvement of the <sup>1</sup>H-MRS data quality and for an increase in the range of reliably quantified metabolites.

## Introduction

Applications of comprehensive metabolic profiling are increasingly common in epidemiology.<sup>1,2</sup> In general, 'metabolic profiling' refers to the simultaneous quantification of a wide range of low-molecular-weight metabolites in a variety of tissues or biofluids. As the fundamental principles of epidemiology require large-scale cohort studies, only minimally invasive, high-throughput screening methods are acceptable for these types of studies. Consequently, metabolic profiling has been almost exclusively applied to biofluids, such as plasma (serum) or urine, using high-resolution liquid-state <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) combined with chromatographic separation.<sup>1</sup> These metabolic profiles provide a top-down 'systems-level' readout of the biochemistry, physiological status and environmental exposure of individuals and populations that can be exploited in personalized medicine and public healthcare.<sup>3</sup> Wide-range metabolic phenotyping has been successfully applied to different health issues, such as nutritional problems,<sup>4</sup> effects of long-term physical activity,<sup>5</sup> type 1 and 2 diabetes<sup>6,7</sup> and cardiovascular diseases.<sup>2</sup> Other studies indicated the potential of blood plasma metabolic phenotyping as a tool to discriminate between cancer types.<sup>8</sup> However, these types of preliminary results must be taken with caution due to the tendency of multivariate classification to overestimate the predictive power of the method when the number of subjects is small.<sup>9</sup> Metabolic phenotyping is becoming important in disease prevention due to its potential to identify individuals or sub-populations at risk, allowing for interventions (change in lifestyle, diet or appropriate medication) before the onset of the disease.<sup>3</sup> Metabolic phenotyping can be further utilized in personalized medicine for patient stratification by analysing pre-dose biofluid metabolite profiles to predict drug effects,<sup>10</sup> which would increase the efficacy of treatment and decrease unwanted side effects.<sup>11,12</sup>

## Metabolic phenotyping and neurological disorders

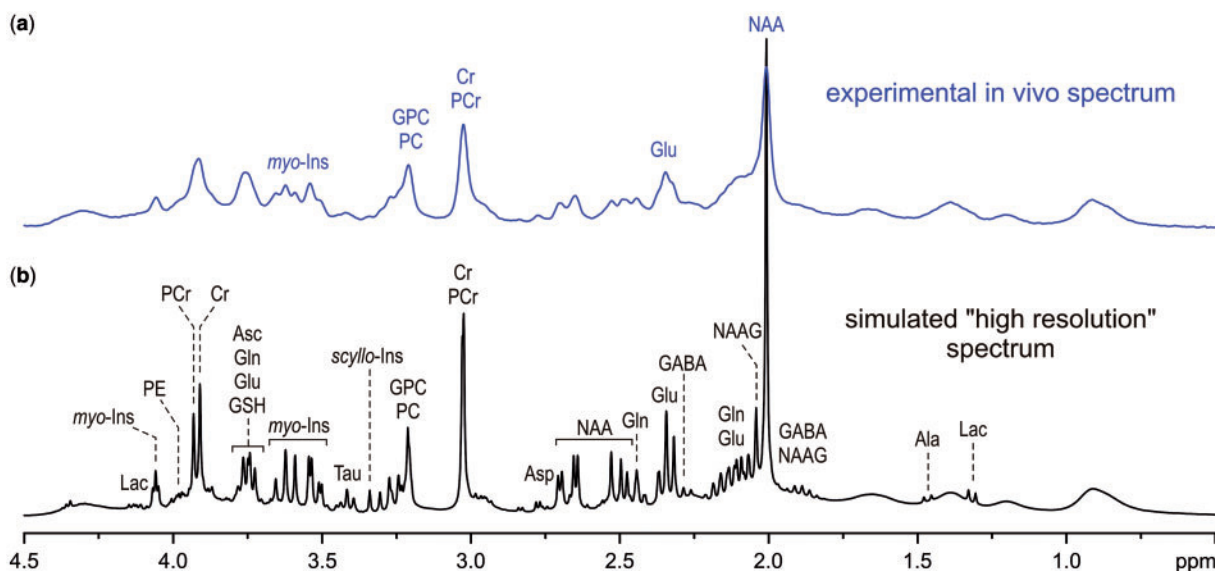
Applications of metabolite phenotyping to neurological disorders are rather sparse.<sup>13,14</sup> The brain metabolism and its homeostasis are well protected from the rest of the body and circulating blood by the blood-brain barrier (BBB), which substantially limits the potential for finding biomarkers for neurological disorders in serum<sup>15</sup> and even more in urine.<sup>16</sup> For that reason, metabolic profiling of cerebrospinal fluid (CSF) should be able to characterize the neurochemical status of the central nervous system (CNS) more directly than the profiling of plasma or urine.<sup>17-19</sup>

However, the CSF sample collection by lumbar puncture is invasive and is justified only for diagnostic purposes. Such an invasive procedure is not ideal for repeated follow-up visits to assess the efficacy of treatment and cannot be applied for screening in the general population.<sup>19</sup> Moreover, metabolic profiling of CSF has a limited capability to map regional metabolic deviations from brain homeostasis due to the high complexity of the CNS and its regional heterogeneity (e.g. cortical and subcortical structures, grey and white matter), diversity of neural cell types (neurons, glia), neuronal morphology (soma, dendrites, axons) and neuronal diversity (e.g. glutamatergic and GABAergic neurons). For these reasons, profiling techniques directly targeting the brain tissue would be preferential. Although *ex vivo* metabolic profiling of intact brain tissue (magic-angle-spinning NMR) or tissue extracts is a powerful tool for animal model studies,<sup>20</sup> sample collection using brain biopsy is totally unsuitable for any type of epidemiological screening in the general population. Considering the pros and cons of previously mentioned methodologies, localized *in vivo* <sup>1</sup>H magnetic resonance spectroscopy (MRS) might be the method of choice for metabolic profiling and phenotyping of CNS diseases.

## Does <sup>1</sup>H-MRS meet the criteria for epidemiology screening?

In epidemiology, prerequisites for metabolomics methodologies are high cost-effectiveness via high-throughput and absolute quantification of molecular measures.<sup>2</sup> Does the current status of neurochemical profiling by *in vivo* <sup>1</sup>H-MRS meet the criteria for molecular epidemiological studies? The answer is NO. However, <sup>1</sup>H-MRS may have the potential to serve as a reliable method for neurochemical profiling in clinical diagnostics as well as for metabolic phenotyping in epidemiologic studies. Our optimism is based on our own experience with <sup>1</sup>H-MRS and on literature that has demonstrated the feasibility of advanced, non-invasive neurochemical profiling in animals and humans.<sup>21-27</sup> In this review, we will first discuss the methodological requirements for MRS data acquisition, MRS data processing and metabolite quantification that are essential for reliable non-invasive neurochemical profiling. Then we will demonstrate the power of <sup>1</sup>H-MRS-based neurochemical profiling by showing some examples of its application in neuroscience. Finally, we will present our vision and propose the necessary steps to establish <sup>1</sup>H-MRS as a method suitable for large-scale neurochemical profiling in epidemiological research.

NMR-based analysis of biofluids clearly demonstrates that the entire metabolic profile, rather than individual metabolites, is more powerful for capturing



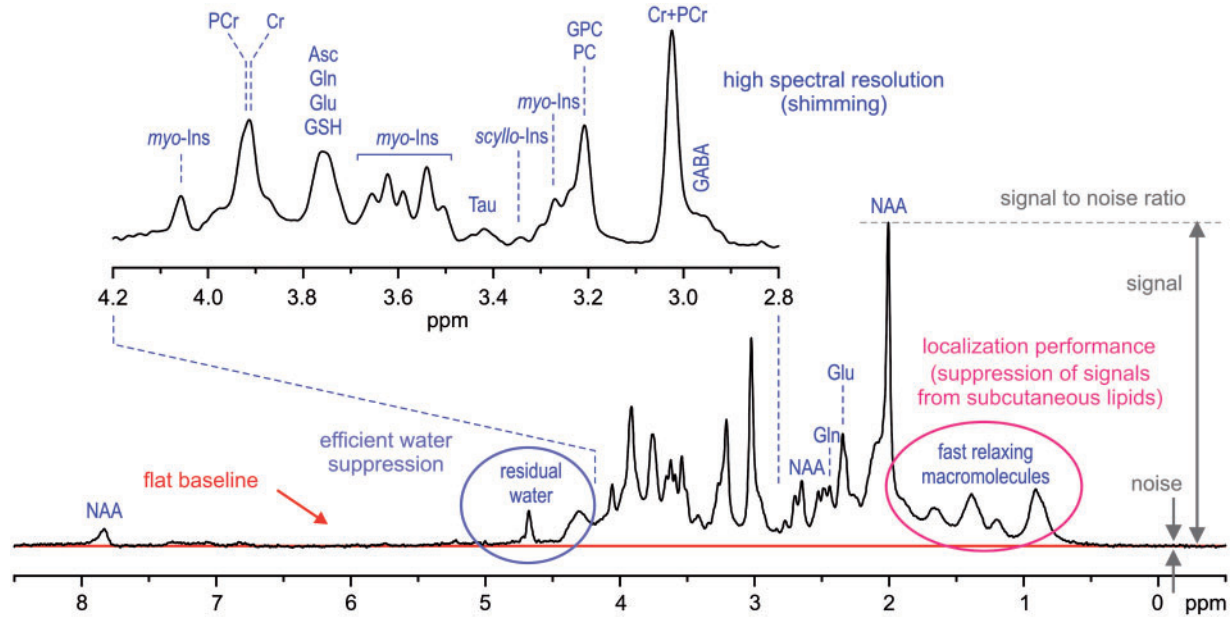
**Figure 1.** Comparison of an experimental *in vivo*  $^1\text{H}$  MR spectrum of the brain achievable at 7 T (a) to a simulated 'high-resolution'  $^1\text{H}$  NMR spectrum (b).

(patho)physiological processes.<sup>2</sup> Thus, meaningful metabolic phenotyping requires a comprehensive quantification of metabolites. Unfortunately, *in vivo*  $^1\text{H}$ -MRS faces several challenges that limit the range of detectable metabolites. One of the key limiting factors is the spectral resolution, which is substantially reduced under *in vivo* conditions relative to liquid  $^1\text{H}$  NMR spectroscopy. This can be seen in Figure 1, where a theoretical human brain spectrum, simulated with a resolution typical for  $^1\text{H}$  NMR spectroscopy of biofluids, is compared with the spectrum experimentally achievable at 7 T. At this reduced spectral resolution, accurate and precise quantification of metabolites is complicated by the strong overlap of their individual spectra. The spectral resolution is not the only challenge for non-invasive metabolite quantification by  $^1\text{H}$ -MRS. In addition, the detection sensitivity of  $^1\text{H}$ -MRS is much lower than the sensitivity typical for  $^1\text{H}$  NMR spectroscopy of biofluids. Furthermore, the volume selection, which is essential for localized  $^1\text{H}$ -MRS, may affect the overall spectral pattern and degrade the spectral quality. Last but not least, metabolite spectra overlap with broad signals of fast-relaxing macromolecules (proteins), which are not possible to eliminate by techniques commonly used for plasma  $^1\text{H}$  NMR spectroscopy<sup>28</sup> due to the short  $T_2$  relaxation times of metabolites and limited RF radio frequency (RF) power for a multi-pulse refocusing train. Consequently, any deterioration in spectral quality, such as increased line width, decreased signal-to-noise ratio (SNR), baseline distortions or contamination by unwanted coherences, has a major impact on the reliability of metabolite quantification. Because of these challenges, the number of metabolites that can

currently be non-invasively quantified in the human brain using  $^1\text{H}$ -MRS routinely available on clinical MRI scanners is limited to three or four: N-acetylaspartate (NAA), total creatine (tCr), total choline (tCho) and occasionally lactate (Lac). It would be rather naive to expect that concentration changes in just these few brain metabolites would be capable of probing the complex network of neurochemical processes. Therefore, improvements in  $^1\text{H}$ -MRS methodology that expand the range of reliably quantifiable metabolites are critical for making this method suitable for metabolic profiling. In the following sections, we will describe basic concepts of advanced  $^1\text{H}$ -MRS methodology (data acquisition, data preprocessing and metabolite quantification) that are critical for extending the range of detectable metabolites and for improving the precision and accuracy of their quantification. For those interested in more technical details of  $^1\text{H}$ -MRS data acquisition and processing, additional text and figures can be found in the [Supplementary data](#), available at *IJE* online.

## Methodology of advanced $^1\text{H}$ -MRS

Similarly to  $^1\text{H}$  NMR spectroscopy of biofluids, high magnetic field strength is also advantageous for *in vivo*  $^1\text{H}$ -MRS because of increased detection sensitivity and chemical shift dispersion at high fields. However, moving to high magnetic fields is not as straightforward for *in vivo* applications because wide-bore magnets for human body MRI/MRS are much more complex and costly than the narrow-bore magnets used for  $^1\text{H}$  NMR spectroscopy of biofluids. In addition, adjustment of the  $B_0$  field



**Figure 2.** Characteristic factors for *in vivo*  $^1\text{H}$ -MR spectra quality assessment (STEAM,  $B_0 = 7\text{ T}$ ,  $TE = 6\text{ ms}$ ,  $TR = 5\text{ s}$ ,  $VOI = 8\text{ ml}$ , number of transients (NT) = 160, grey-matter-rich occipital cortex).

homogeneity ( $B_0$  shimming) and the localization performance of MRS techniques are much more challenging at high fields. Accordingly, localized *in vivo*  $^1\text{H}$  MRS of the brain can only benefit from high magnetic field when these challenges are successfully resolved. Consequently, *in vivo*  $^1\text{H}$ -MRS of the human brain is typically limited to 7 T for research applications and only to 3 T in clinical practice. It should be emphasized that the term ‘ultra-high field’ (UHF) is used differently in analytical chemistry and MRI. This term is typically reserved for magnetic fields  $B_0 > 20\text{ T}$  in NMR spectroscopy of liquids, whereas it is commonly used for human body MRI/MRS at 7 T. Even 3T MRI scanners are considered high-field systems in clinical practice.

The first and utmost requirement for *in vivo* neurochemical profiling by  $^1\text{H}$ -MRS is high quality of the acquired spectra (Figure 2). Spectral resolution is the key factor of the spectral quality and directly affects the reliability of metabolite quantification. A resolution typical for  $^1\text{H}$  NMR spectroscopy of biofluids is not achievable by  $^1\text{H}$ -MRS due to the line broadening caused by microscopic  $B_0$  field heterogeneity on a cellular and microvascular level. Therefore, additional line broadening caused by macroscopic  $B_0$  inhomogeneity within the selected volume of interest (VOI) is not acceptable and has to be eliminated by  $B_0$  shimming. Successful  $B_0$  shimming requires an efficient  $B_0$  mapping technique, such as FASTMAP<sup>29,30</sup> and a powerful  $B_0$  shimming system (shim coils and drivers).

Volume selection is a fundamental feature of *in vivo*  $^1\text{H}$ -MRS data-acquisition techniques, which makes it

significantly different from high-resolution  $^1\text{H}$  NMR spectroscopy of liquids. In theory, the  $^1\text{H}$ -MR spectrum exclusively originates from the selected VOI without any contamination from the surrounding tissue. These unwanted coherences, especially from subcutaneous lipids (1–2 ppm chemical shift range), can significantly degrade the spectral quality (Figure 2). In addition, volume selection at high fields is challenged by the chemical shift displacement error (CSDE), i.e. spatial displacement of volumes for off-resonance signals from the prescribed VOI (see more detailed explanation in the [Supplementary data](#), available at *IJE* online). Last, but not least, high spectral quality requires highly efficient water suppression (Figure 2) because the residual water signal may cause major distortions of spectrum baseline and overlap with surrounding metabolite signals. Different localization strategies have been successfully utilized in pulse sequences developed for ultra-high magnetic fields<sup>23,25,31–34</sup> in order to improve the localization performance and, consequently, to provide spectra of the best quality. Each of these techniques has its pros and cons, but it appears that the semi-LASER localization sequence<sup>33</sup> combined with VAPOR water suppression<sup>25,34</sup> is currently the best compromise for operational feasibility and performance. Despite excellent  $B_0$  shimming and high-performance localization, the final spectral quality can be severely compromised by frequency and phase fluctuations induced by physiological motion (respiratory and cardiac cycles) during data collection. For these reasons, a single scan averaging mode (where each individual scan is stored separately) is highly advantageous for *in vivo*

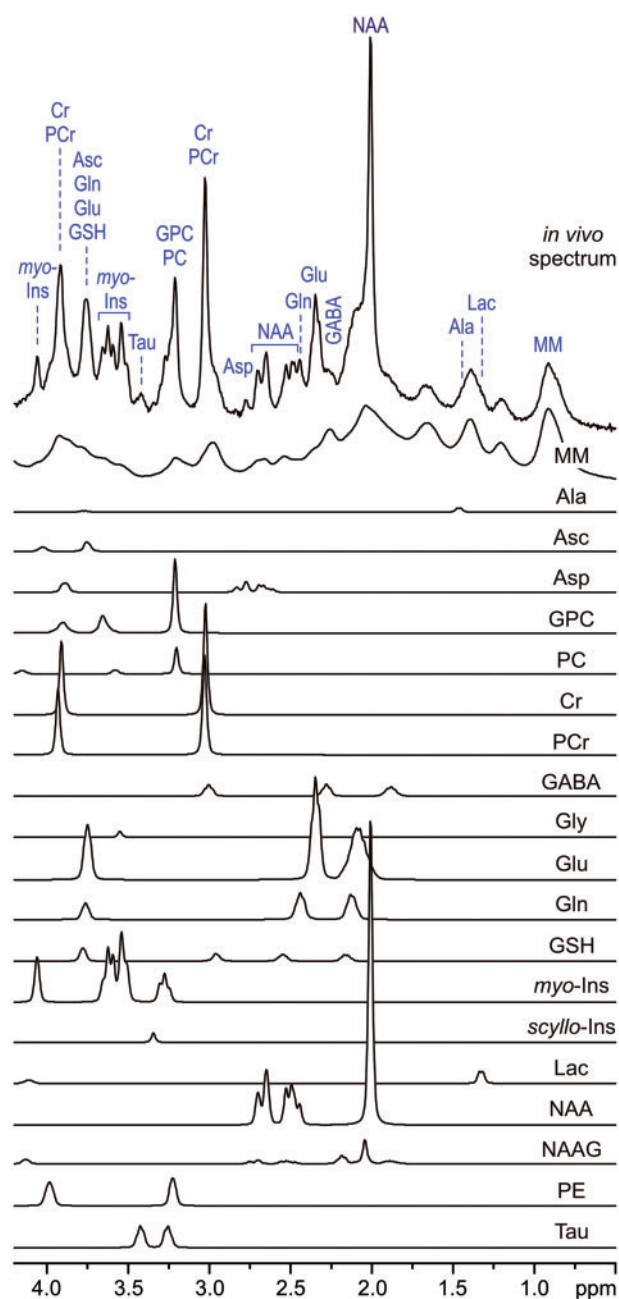


applications because it allows for frequency and phase correction of individual scans and elimination of uncorrectable scans before summation. This approach helps to maintain the best achievable spectral quality (Figure 2).

### Metabolite quantification

The natural (intrinsic) linewidth of *in vivo*  $^1\text{H}$ -MR spectra increases with the magnetic field strength due to shortened metabolite  $T_2$  relaxation times and microscopic  $B_0$  field inhomogeneity.<sup>35,36</sup> Consequently, the overlap of metabolite spectra cannot be eliminated even at UHF, despite increased chemical shift dispersion. Moreover, metabolite spectra overlap with underlying broad signals of fast-relaxing macromolecules, which makes the quantification even more challenging. Fortunately, the range of metabolites that contribute dominantly to brain spectra is typically known. Except in some rare metabolic diseases, the concentrations of only about 20–25 metabolites reach the current  $^1\text{H}$ -MRS detection threshold of  $\sim 0.4 \mu\text{mol/g}$ . The primary goal of  $^1\text{H}$ -MRS is precise and accurate quantification of this well-defined set of metabolites detectable in the brain. The  $^1\text{H}$  MR spectra of these metabolites, which are used as a prior knowledge, are essential for a meaningful quantification of *in vivo*  $^1\text{H}$ -MRS data. Although metabolite  $^1\text{H}$  MR spectra can be measured experimentally ( $T = 37^\circ\text{C}$ ,  $\text{pH} = 7.2$ ), the simulation of these spectra using published chemical shifts and J-couplings of brain metabolites<sup>37</sup> is recently preferred. The commonly used fitting programmes for  $^1\text{H}$ -MRS data analysis work in either the time domain (jMRUI<sup>38,39</sup>) or the frequency domain (LCModel<sup>40</sup>). Figure 3 shows the basic principle of the LCModel analysis of a  $^1\text{H}$  MR spectrum acquired from the human brain at 7 T. The experimental *in vivo* spectrum is modelled as a linear combination of metabolite spectra from the LCModel basis set. This basis set also includes the spectrum of fast-relaxing macromolecules, which was measured by the metabolite nulling technique using an inversion-recovery experiment.<sup>24–26</sup> The inclusion of the macromolecule spectrum into the basis set significantly improves the robustness of metabolite quantification, especially for metabolites that are present at low concentrations and whose spectra overlap with other strong signals, such as ascorbate (Asc),  $\gamma$ -aminobutyric acid (GABA) or glutathione (GSH). Quantification errors are estimated by Cramér-Rao lower bounds (CRLB).

*In vivo* metabolite quantification requires appropriate referencing. The signal of total creatine has been widely used as an internal reference. However, this approach does not fulfil the criteria for ‘absolute’ quantification, because the tCr content differs between brain regions<sup>22</sup> and



**Figure 3.** LCModel analysis of an *in vivo*  $^1\text{H}$  MR spectrum acquired from the human brain at 7 T (STEAM,  $TE = 6 \text{ ms}$ ,  $TR = 5 \text{ s}$ ,  $NT = 160$ , grey-matter-rich occipital cortex). *In vivo* spectrum can be modelled as a linear combination of brain metabolite spectra from the LCModel basis set: macromolecules (MM), alanine (Ala), ascorbate (Asc), glycerophosphocholine (GPC), phosphocholine (PC), creatine (Cr), phosphocreatine (PCr),  $\gamma$ -aminobutyric acid (GABA), glycine (Gly), glutamate (Glu), glutamine (Gln), glutathione (GSH), *myo*-inositol (*myo*-Ins), *scyllo*-inositol (*scyllo*-Ins), lactate (Lac), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau). Modified with permission from Tkac *et al.*<sup>25</sup> © 2005 Springer.

between grey and white matter<sup>41</sup>, and may change due to neurodegenerative processes.<sup>42</sup> Using unsuppressed water signal as a reference appears to be the best available approximation for metabolite quantification because it is

robust and only requires the knowledge of the brain tissue water content.

Finally,  $T_1$  and  $T_2$  relaxation effects have to be taken into account for 'absolute' metabolite quantification. Correcting for relaxation effects requires knowledge of  $T_1$  and  $T_2$ , but it is rather difficult to measure them precisely.<sup>43,44</sup> In addition, these values vary across different brain regions and with age.<sup>43,44</sup> Contrary to metabolite  $T_1$  relaxation times, which are nearly independent of the field strength,  $T_2$  relaxation times become significantly shorter with increasing  $B_0$  field.<sup>35</sup> This shortening of  $T_2$  values leads to a major loss in signal intensity if long echo-times (TEs) are used at high fields. Moreover, signals of metabolites with spin-spin coupled protons (e.g. glutamate, glutamine, GABA) attenuate at longer TEs because of destructive interferences caused by the J-evolution. Therefore, neurochemical profiling strictly requires localization sequences with short TEs because the substantial part of the neurochemical information available at short TEs is irreversibly lost at long TEs. The 'absolute' metabolite quantification can be substantially simplified by acquiring data using an ultra-short TE localization sequence with a long repetition time (TR), when relaxation effects become negligible and can be ignored. The STEAM sequence is the method of choice for ultra-short TE localization, as it allows a reduction of the TE to 6 ms.<sup>25,26,36</sup>

### Advanced $^1\text{H}$ -MRS neurochemical profiling at 7 T

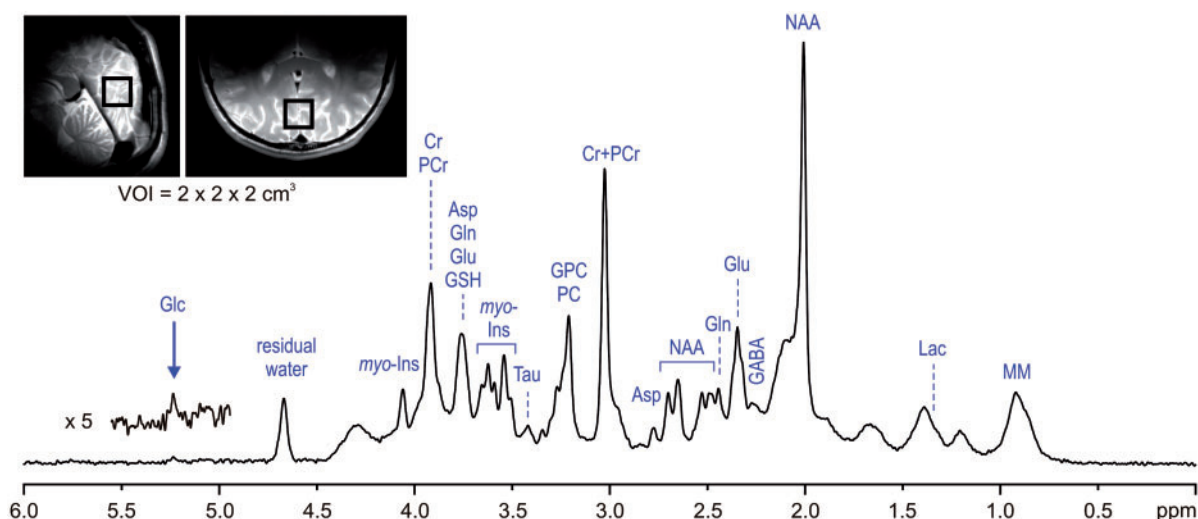
The following paragraph demonstrates the potential and limitations of non-invasive neurochemical profiling using the most advanced methodologies currently available for  $^1\text{H}$ -MRS at ultra-high magnetic fields. An ultra-short echo-time STEAM sequence with interleaved OVS and VAPOR water suppression<sup>25,36</sup> has an excellent localization performance and, combined with FASTMAP  $B_0$  shimming, can provide high-quality *in vivo*  $^1\text{H}$  MR spectra from the human brain at 7 T (Figure 4). This type of artefact-free spectra with the best available spectral resolution is the critical requirement for reliable neurochemical profiling. This method was used in a study comparing neurochemical profiling at 4 T and 7 T.<sup>26</sup> The results of this study clearly demonstrate that spectra of high quality can be routinely achieved (Figure 5a). The neurochemical profiles from both 4 T and 7 T, quantified from the grey-matter-rich occipital lobe of the same group of subjects, were in very good agreement (Figure 5b). Surprisingly, standard deviations (SDs) of quantified metabolite concentrations were not lower at 7 T than at 4 T. The similarity of SDs indicated that the precision in metabolite quantification achieved at both field strengths was already better than the

variation in metabolite levels between subjects. This ability of  $^1\text{H}$ -MRS to map the actual inter-subject variability in metabolite concentration in a specific brain region was demonstrated by a strong correlation between metabolite levels of individual subjects determined at 4 T and 7 T (Figure 6).

Similar results were found in a recent multi-centre study that focused on the accuracy and reproducibility of neurochemical profiles in the human brain at 7 T.<sup>27</sup> This study was performed at four different institutions (University Duisburg-Essen, University of Minnesota, Leiden University Medical Center and University Medical Center Utrecht) using 7T MRI scanners from two different vendors (Philips or Siemens). The same seven subjects were each examined twice at each site using the same protocol: FASTMAP  $B_0$  shimming, semi-LASER localization sequence<sup>33</sup> combined with VAPOR water suppression,<sup>25,34</sup> LCModel metabolite quantification.  $^1\text{H}$ -MRS data were acquired from the grey-matter-rich posterior cingulate cortex and the white-matter-rich corona radiata. Concentrations of 10 metabolites were consistently quantified with CRLB < 20%. Neurochemical profiles measured at different sites equipped with MRI scanners from two different vendors were in reasonably good agreement. The test-retest measurements showed a high level of reproducibility and, for several metabolites, such as glutamate (Glu), tCr, tCho and *myo*-inositol, the primary source of variance was found between subjects. This study clearly demonstrates that harmonization of data acquisition and post-processing of  $^1\text{H}$ -MRS can produce very similar results at four different 7T MRI sites.

$^1\text{H}$ -MRS can measure regional differences between neurochemical profiles in the human brain.<sup>22</sup> The neurochemical profiles measured from the brain regions that are of interest for various neurological disorders (frontal white matter, posterior cingulate, putamen, substantia nigra, pons and cerebellar vermis) show noticeable differences (Figure 7). Because of these regional differences in neurochemical profiles, highly consistent and reproducible VOI placement is extremely important in order to minimize variations in quantified metabolite levels. In addition, Figure 7 clearly demonstrates that using tCr as a universal internal reference for 'absolute' metabolite quantification is not appropriate.

The current capabilities of advanced  $^1\text{H}$ -MRS at 7 T can be nicely demonstrated by studies that aimed to measure neurochemical changes in the activated human brain.<sup>45-49</sup> These studies clearly illustrate that the advanced technology and methodology of  $^1\text{H}$ -MRS currently available has the power to detect metabolite concentration changes as low as 0.2  $\mu\text{mol/g}$ . Visual stimulation studies<sup>45-48</sup> show very consistent results of small, but



**Figure 4.** *In vivo*  $^1\text{H}$ -MR spectrum acquired from the human brain at 7 T (STEAM, TE = 6 ms, TR = 5 s, VOI = 8 ml, NT = 160). Modified with permission from Tkac *et al.*<sup>25</sup> © 2005 Springer.

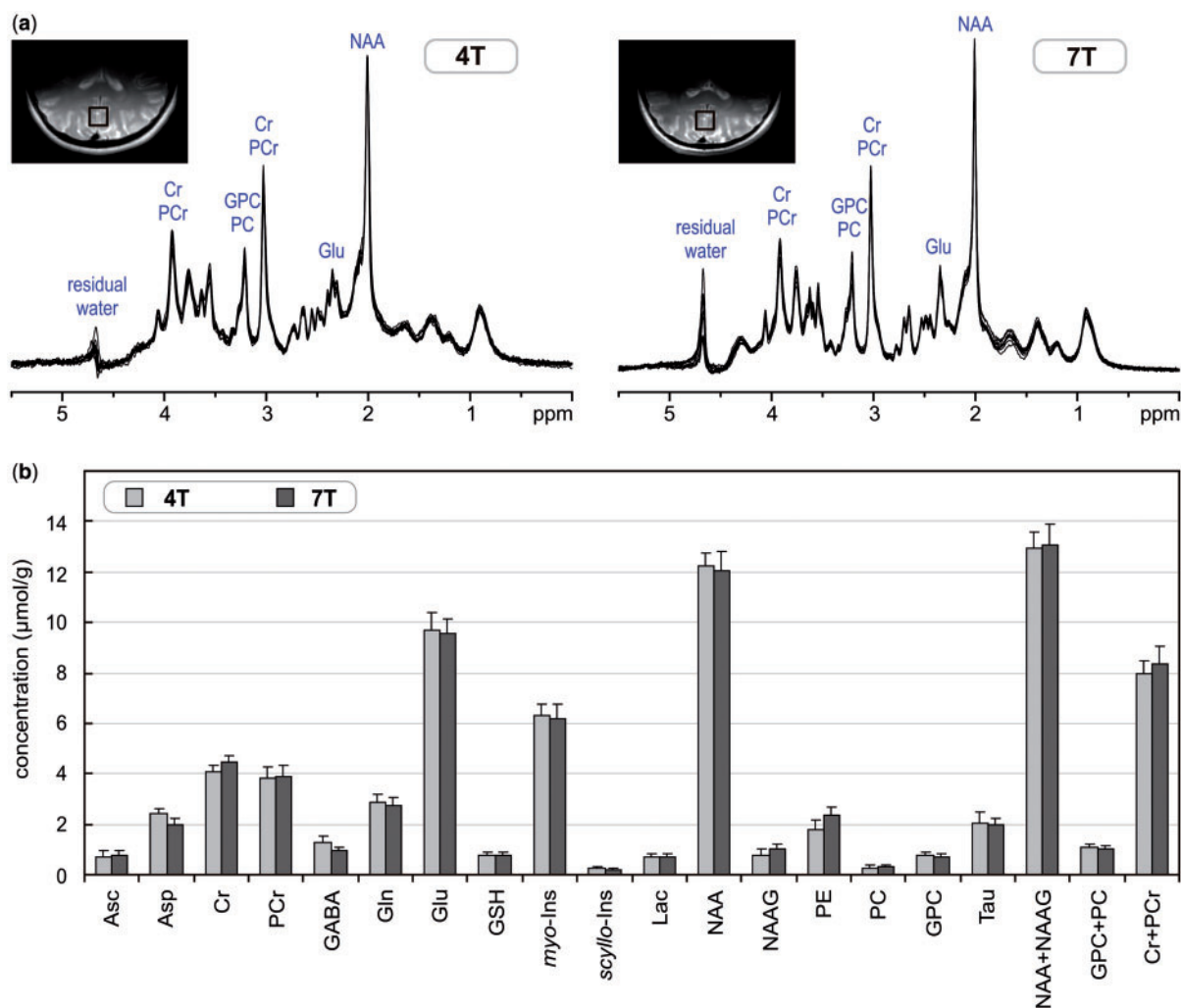
significant, changes in four metabolites (Lac, Glu, glucose, aspartate) that are directly linked to energy metabolism. Techniques used in these studies provide neurochemical profiles with up to 19 brain metabolites and enable quantification of their changes during stimulation paradigm with precision better than  $0.1 \mu\text{mol/g}$ .<sup>45,46,48</sup>

### Feasibility of $^1\text{H}$ -MRS neurochemical profiling at 3 T

Although 1.5 T is the most common field strength for clinical MRI (approximately 15 000 units already installed and more than 50% of 2500 new units sold worldwide), the sensitivity and spectral resolution at this field are not sufficient for a meaningful neurochemical profiling (i.e. reliable quantification of metabolites beyond NAA, tCr and tCh). An ultra-high magnetic field is better-suited for non-invasive neurochemical profiling, but widespread availability of 7T MRI scanners for large-scale cohort studies is not realistic in the near future. For that reason, 3T MRI scanners, which are increasingly available on clinical sites, seem to be a feasible platform for neurochemical profiling. Although slow implementation of newly developed  $^1\text{H}$ -MRS techniques on clinical MRI scanners has created a widening gap between what is feasible at specialized MR research centres and what is currently available for routine clinical practice, advanced  $^1\text{H}$ -MRS methodology developed for 7 T can be implemented on 3T MRI scanners without any requirement on hardware upgrades. It was already successfully demonstrated that simply upgrading the pulse sequence (software), which consisted of FASTMAP shimming<sup>29,30</sup> and semi-LASER localization<sup>33</sup> with VAPOR water suppression,<sup>25,34</sup> significantly improved the

$^1\text{H}$ -MRS data quality and increased the range of reliably quantified metabolites from the original 3–5 (using vendor-provided sequences) up to 13.<sup>21</sup> In addition, the same methodology was implemented at two different institutions (the University of Minnesota, in Minneapolis, and the Institut du Cerveau et de la Moelle, in Paris) and the measured neurochemical profiles (vermis, ponds) were nearly identical between these sites. Moreover, this approach showed the feasibility to detect inter-individual differences in the healthy brain.<sup>21</sup>

A similar approach was used to investigate the feasibility and reproducibility of neurochemical profiling in the human hippocampus at 3 T.<sup>43,50</sup> The hippocampus is a brain region of high clinical interest because its dysfunction is associated with several neurological and neuropsychiatric disorders such as Alzheimer's disease, epilepsy, schizophrenia and depression. However,  $^1\text{H}$ -MRS of the hippocampus is challenging due to its size and shape and the severe  $B_0$  inhomogeneity in that brain region. Using a semi-LASER localization sequence and FASTMAP shimming routinely provided  $^1\text{H}$ -MR spectra of high quality from a relatively small VOI of  $\sim 4 \text{ ml}$ .<sup>50</sup> Achieved spectral quality allowed reliable quantification of up to 12 brain metabolites. This study clearly demonstrates that neurochemical profiling of the hippocampus is feasible at 3 T despite technical challenges. For most metabolites, the between-session variation was lower than between-subject variation, indicating that the method has the sensitivity to detect inter-individual differences in the healthy brain. Our preliminary  $^1\text{H}$ -MRS results from the occipital cortex demonstrate that it is even possible to increase the number of quantifiable metabolites at 3 T close to the range achievable at 7 T, but it requires exceptional spectral quality (see



**Figure 5.** (a) Superposition of *in vivo* <sup>1</sup>H-MR spectra acquired from the grey-matter-rich occipital cortex of 10 healthy subjects at 4 T and 7 T (STEAM, VOI = 8 ml, NT = 160, TE = 4 ms at 4 T and TE = 6 ms at 7 T). (b) Comparison of neurochemical profiles determined from these 4T and 7T spectra. Error bars indicate SD. Only concentrations of Asp, GABA, NAAG and PE were statistically different between 4 T and 7 T. Modified with permission from Tkac *et al.*<sup>26</sup> © 2009 John Wiley and Sons.

Supplementary Figures 1 and 2, available as Supplementary data at *IJE* online).

### Applications of <sup>1</sup>H-MRS for CNS disorders

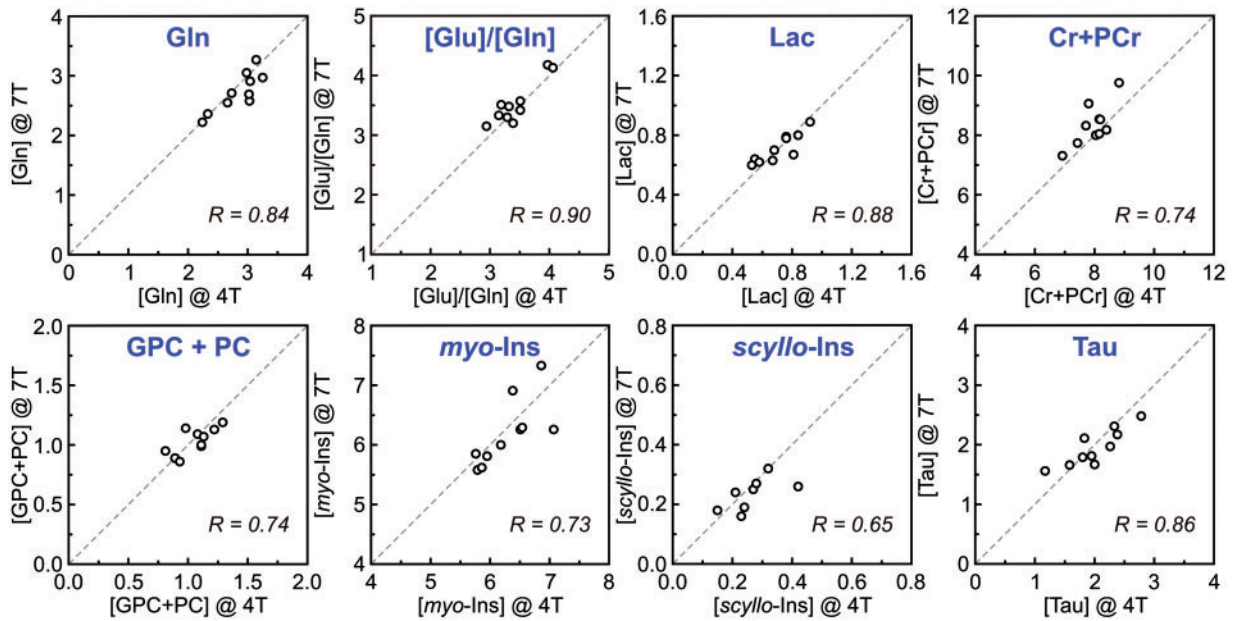
*In vivo* <sup>1</sup>H-MRS has been widely applied to investigate CNS disorders. A PubMed search for keywords 'MR spectroscopy human CNS disorders' retrieved over 6500 original papers and 1500 reviews. These MRS studies report valuable observations about a variety of CNS disorders, but the range of detectable metabolites is almost completely limited only to NAA, tCr and tCho. Some recent publications of interest include reviews on Alzheimer disease and mild cognitive impairments,<sup>51,52</sup> common dementias,<sup>53</sup> psychiatric disorders,<sup>54</sup> autism spectrum disorders<sup>55</sup> and alcohol abuse disorders.<sup>56</sup> Recently, the MRS Consensus Group published useful recommendations for

clinical <sup>1</sup>H-MRS in CNS disorders.<sup>57</sup> However, there are few published studies utilizing the true potential of <sup>1</sup>H-MRS-based neurochemical profiling to investigate metabolic changes associated with the brain disorders. For example, *in vivo* neurochemical profiling was used to study biochemical changes in patients with spinocerebellar ataxias,<sup>58–61</sup> type 1 diabetes<sup>62</sup> and adrenoleukodystrophy.<sup>63</sup>

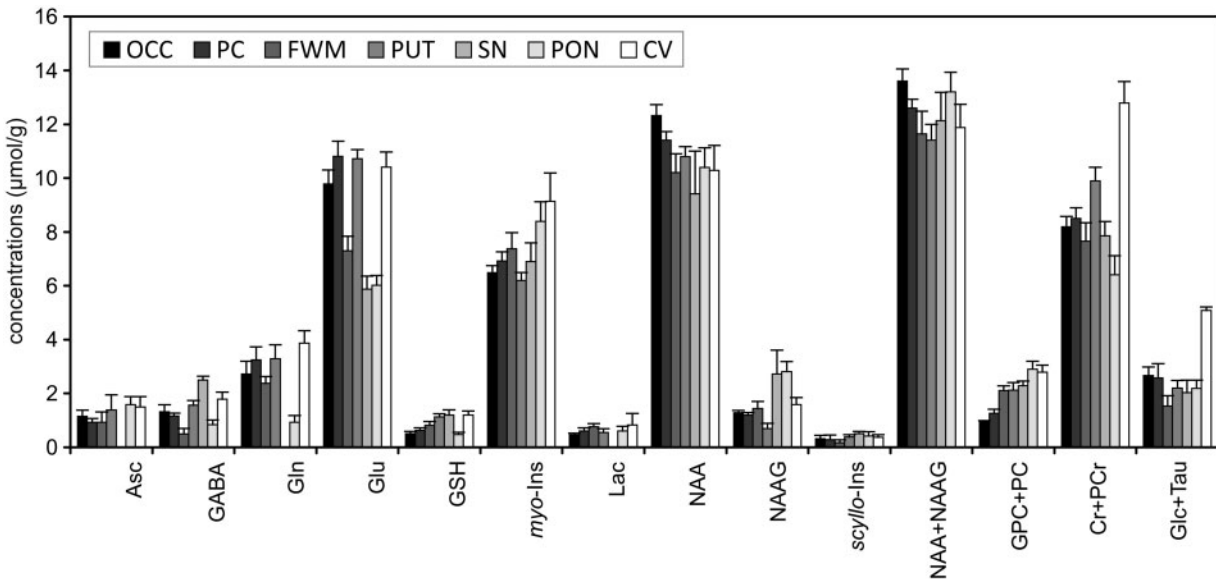
### Potential of *in vivo* neurochemical profiling in epidemiology

As stated in the previous section of this paper, non-invasive neurochemical profiling is, in principle, feasible on 3T MRI scanners that are commonly available in hospitals. However, successful neurochemical profiling by <sup>1</sup>H-MRS requires the implementation of efficient B<sub>0</sub> shimming





**Figure 6.** Correlation between metabolite concentrations and concentration ratios of individual subjects determined from 4T and 7T <sup>1</sup>H-MR spectra. Concentrations are expressed in  $\mu\text{mol/g}$ . Modified with permission from Tkac *et al.*<sup>26</sup> © 2009 John Wiley and Sons.



**Figure 7.** Comparison of neurochemical profiles quantified from different regions in the human brain at 7 T. STEAM, TE = 8, TR = 5 s, N = 5. Error bars indicate SD. Significant regional differences were found for all metabolites except Lac and *scyllo*-Ins (one-way analysis of variance with Tukey *post hoc*,  $p < 0.005$ ). Brain regions: occipital cortex (OCC), posterior cingulate (PC), frontal white matter (FWM), putamen (PUT), substantia nigra (SN), pons (PON), cerebellar vermis (CV). Modified with permission from Emir *et al.*<sup>22</sup> © 2012 John Wiley and Sons.

methods (e.g. FASTMAP) and high-performance localization techniques (e.g. semi-LASER, VAPOR water suppression). In addition, preprocessing software and appropriate metabolite quantification technique (e.g. LCModel), with a database of well-constructed prior knowledge, are also required. The main problem for wider use of <sup>1</sup>H-MRS for screening purposes is the duration of the scanning session and consequently the cost of this examination. The

duration of a typical <sup>1</sup>H-MRS scanning session, including MRI for positioning of the VOI, might be as long as 60 min, which is far too long for screening. Using a fully automated approach, it should be possible to reduce the time period necessary for parameter optimization ( $B_0$  shimming, power adjustment, water suppression) from about 10 min to 2 min or less. With 5 min of <sup>1</sup>H-MRS data acquisition and an additional 3 min for structural MRI,

reduction of the scanning time below 10 min seems to be feasible. Such an approach would significantly increase the throughput of the method and thereby reduce the cost of examination. Although higher throughput for  $^1\text{H}$ -MRS-based screening is desirable, any improvements in the throughput cannot come at the expense of the overall spectral quality.

As neurochemical profiles show significant regional differences (Figure 7), placement of the VOI plays an important role in data reproducibility. For this reason, automatic VOI positioning methods have been developed for  $^1\text{H}$ -MRS.<sup>64</sup> These regional differences in metabolite concentrations further complicate  $^1\text{H}$ -MRS by making it challenging to choose the most appropriate brain region for neurochemical profiling. From this viewpoint, comprehensive neurochemical profiling of multiple brain regions would be preferential but would substantially decrease the throughput. Thus, it appears that MR spectroscopic imaging (MRSI), a non-invasive technique that provides information about the spatial distribution of metabolites, might be the method of choice for a potential non-invasive neurochemical profiling. However, even for the most advanced MRSI techniques currently available,<sup>65,66</sup> the range of quantifiable metabolites and the precision of their quantification are behind the capabilities of single voxel MRS techniques.

Finally, recently published  $^1\text{H}$ -MRS trials clearly demonstrated that it is feasible to obtain highly reproducible data at different sites (in the USA and Europe) using MRI scanners from different vendors.<sup>27</sup> This was possible by implementing the same MRS methodology including data acquisition and processing (FASTMAP shimming, semi-LASER localization sequence, VAPOR water suppression, LCModel processing). There is an ongoing multi-centre effort (NIH grant: Partnership for MRS biomarker development, PI: Gülin Öz, University of Minnesota) that seeks to simplify the operator input necessary to acquire MRS data, to make it robust and user-friendly and to make it available for all major clinical MR platforms. This effort will enhance the possibility to make high-quality neurochemical profiling widely available on 3T clinical MRI scanners, which will increase the probability for successful *in vivo* large-scale cohort screening in the near future.

In conclusion, *in vivo* neurochemical profiling in the human brain is feasible, but the widespread use requires the implementation of the most advanced methodology of  $^1\text{H}$ -MRS, including  $B_0$  shimming, localization pulse sequences, data processing and metabolite quantification. In addition, these techniques have to be broadly available on 3T MRI scanners from major MR systems vendors (Siemens, Philips and GE). Easy access to *in vivo* neurochemical profiling for the general population is

probably not feasible in the near future, but this screening technique should be made available as soon as possible for disease prevention, at least for at-risk sub-populations.

## Supplementary Data

Supplementary data are available at *IJE* online.

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