Electronic reference for absolute quantification of brain metabolites by $^1$H-MRS on clinical whole-body imaging

Référence électronique pour la quantification absolue des métabolites cérébraux en spectroscopie protonique sur un imageur clinique

H. Desal$^a$,* N. Pineda Alonso$^b$, S. Akoka$^b$

$^a$ Service de neuroradiologie diagnostique et interventionnelle, hôpital Guillaume et René-Laennec, CHU de Nantes, boulevard Jacques-Monod—St-Herblain, 44093 Nantes cedex 1, France
$^b$ UMR CNRS n° 6230, chimie et interdisciplinarité : synthèse, analyse, modélisation (CEISAM), université de Nantes, 2, rue de-la-Houssinière, BP 92208, 44322 Nantes cedex 03, France

Available online 23 March 2010

KEYWORDS
$^1$H-MRS; ERETIC; Quantification

Summary
Background and purpose. — The electronic reference to access in vivo concentrations (ERETIC) method is a promising technique for absolute concentration quantification by brain proton magnetic resonance spectroscopy ($^1$H-MRS). However, in its usual form, the technique cannot be implemented in most clinical MR scanners. For this reason, we propose a new strategy for transmitting the ERETIC signal before localized spectroscopy acquisition, thereby allowing its use in clinical MR scanners.

Methods. — ERETIC signal acquisition, using a dedicated sequence, was carried out immediately before the MR sequence. This approach was evaluated on phantoms of known metabolite concentrations and in 10 healthy volunteers. The results were then compared with those obtained using the water signal as reference.

Results. — Measurements in vitro showed that the standard deviations measured by the ERETIC method were similar to those using the water-signal reference method. Also, values for metabolite concentrations in vivo were in good agreement with those found in the literature for normal white matter in human brains. Concentrations obtained by ERETIC showed good linear correlation compared with the values obtained by the water-signal reference method.

Conclusion. — Our preliminary study shows that the ERETIC method appears to be a reliable technique that can overcome most of the drawbacks observed with other absolute quantification methods. However, further studies involving larger patient groups are needed to confirm these findings.

© 2010 Elsevier Masson SAS. All rights reserved.
Introduction

Although magnetic resonance spectroscopy (MRS) is a powerful diagnostic tool that has been routinely used for more than 60 years in the fields of physics, chemistry and biochemistry, its role in medicine has remained limited [1]. One reason for this could be that relative concentrations are more often used, as they have the advantage of being easily applied. However, the drawback of such an approach is that no reliable values can be obtained. Furthermore, if both peaks increase in proportion, the stability of the ratio may disguise a disease process [2].

For this reason, it is essential to isolate quantitative information from each peak. As the peak area is proportional to the concentration of the corresponding metabolite, the individual and absolute concentrations can be estimated if at least one concentration is known (and used as a reference concentration).

Many strategies of absolute quantification have been proposed [3], including electronic reference to access in vivo concentrations (ERETIC) [4], which is a promising avenue of research. The method has already proven its effectiveness in several applications [5—7]. However, in its original form [4], the ERETIC method assumes the emission of an electronic signal with all the characteristics of a nuclear magnetic resonance (NMR) signal to produce an additional peak in the spectrum. This suggests that the ERETIC signal is transmitted during the reception of the NMR signal, making it necessary to use a second radiofrequency (RF) channel capable of transmitting while the principal channel is in reception mode.

This means that ERETIC can easily be implemented on scanners equipped with at least two RF channels in their basic configuration. However, most clinical scanners have only one such channel.

To overcome this problem, we have developed an approach that involves transmitting the ERETIC signal either immediately before or just after the localized spectroscopy acquisition, as was previously proposed for high-resolution NMR [8]. This novel approach has been evaluated using phantoms of known metabolite concentrations in healthy volunteers.

Patients and methods

Volunteers

The investigation complied with Health Insurance Portability and Accountability Act (HIPAA), and was approved by our institution’s internal review board. Informed consent was obtained from the 10 healthy volunteers (four women and six men) included in the study. They ranged in age from 21 to 47 years (mean 33.5 years), and had no history of neurological symptoms; all had normal MRI results.

Phantoms

Phantom A was a 2.5-L sphere; the solution was prepared by dissolving choline in 100 mM of phosphate buffer with NaN₃ (0.1%) for sterility. A 0.5-M dose of gadolinium-tetraazacyclododecane-tetraacetic acid (Gd-DOTA, 1%; Dotarem; Guerbet, Paris) was added to obtain relaxation times close to those of the human brain. Phantom B was a 100-mL sphere; the solution was prepared by dissolving N-acetylaspargate (NAA), creatine, myoinositol, glutamate, choline and deuterated water in 100 mM of phosphate buffer with NaN₃ (0.1%) for sterility. Gd-DOTA 0.5M (1%) was also added. In both phantoms, the pH was adjusted to 7.2 ± 0.02.

High-resolution MRS measurements

Metabolite and water concentrations were measured with high accuracy in both phantoms using a 500-MHz high-resolution spectrometer (DRX 500; Bruker Biospin SA, Wissembourg, France). All measurements were carried out five times to confirm reproducibility (Table 1).

MRS acquisition

All measurements were made using a 1.5-T whole-body MRI system (Sonata; Siemens AG, Erlangen, Germany) with a circularly polarized head coil. Images for localization were acquired with TrueFISP 2D sequences. Water-suppressed and water ¹H-MRS were performed, using double spin-echo (SE) point-resolved spectroscopy (PRESS) sequences, with an echo time (TE) of 30 ms and a repetition time (TR) of 3000 ms. The number of signal accumulations was 96 for the water-suppressed spectra and two for the water spectra in both volunteers and phantoms. The sampling frequency was 1100 Hz, and 1024 complex sampling points were acquired. Voxel size was 20 x 20 x 20 mm in the white matter of the left frontal region. All adjustments and shimming were made by the automatic routine of the scanner. The width at the half-maximum height of the water resonance line was always less than 5 Hz after line broadening. Measurements of the phantoms were made five times to allow assessment of reproducibility in the clinical scanner.

The ERETIC signal

The ERETIC and NMR signals were not acquired simultaneously. ERETIC signal acquisition was performed, using a dedicated sequence, immediately before the MR sequence. As the ERETIC RF signal cannot be spatially localized (as in a classical spectroscopic sequence), we obtained the ERETIC sequence from a free induction decay (FID) sequence. However, instead of transmitting a high-power RF pulse before the signal-detection period, the ERETIC sequence sent a rectangular-shaped pulse, of 5 ms duration, during the sampling period. This pulse was sent out 5 ms after the onset of acquisition (Fig. 1A) and was transmitted by the body coil. Pulse amplitude was determined as usual by the transmission reference voltage and, therefore, can be set by the protocol.

To detect the ERETIC pulse with the coil in its usual loaded state, it was necessary to prevent detuning the head coil, a state that had to be maintained throughout the entire experiment.
Table 1  Concentrations ± SD (mM) measured on phantoms calculated for five measurements.

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>NAA</th>
<th>Choline</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR NMR</td>
<td>39.8 ± 0.1</td>
<td>11.49 ± 0.09</td>
<td>2.57 ± 0.01</td>
<td>8.27 ± 0.05</td>
</tr>
<tr>
<td>Ref Water</td>
<td>11.0 ± 0.2</td>
<td>2.7 ± 0.8</td>
<td>7.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Ref ERETIC</td>
<td>39.7 ± 0.3</td>
<td>11.1 ± 0.1</td>
<td>2.7 ± 0.5</td>
<td>7.6 ± 0.2</td>
</tr>
</tbody>
</table>

NAA: N-acetylaspartate; HR NMR: concentration measured by high-resolution NMR; Ref Water: concentration measured using water as reference; Ref ERETIC: concentration measured using ERETIC signal as reference.

Only one scan was acquired for each ERETIC acquisition. The sampling frequency was 1100 Hz, and 1024 complex sample points were acquired.

MR data processing

The spectra from both phantoms and volunteers were processed as follows: before the Fourier transform, an exponential filter centered at 0 ms, with a width of 200 ms, was applied, and all the data were zero-filled to 2048 points. Automatic phase correction at 0 order, and baseline correction at first order, were also applied. Spectra were processed in the frequency domain. An iterative least-squares fitted algorithm adjusted the spectrum to a linear combination of simulated multiplets (Fig. 2) [9].

The ERETIC signal was separately processed. After the Fourier transform (Fig. 1C), post-processing was done in the frequency domain in the same way as with the other spectra, using the spectroscopy software of our system. Processing the ERETIC signal in the temporal domain is also possible. However, the analysis was based on the frequency domain, using the spectroscopy post-processing package of our MRI system.

Normalization of peak areas

The ERETIC signal received is represented by the formula \( S_{\text{ERET}} = S_{\text{ERET}}^0 \cdot f_r \cdot f_c \cdot k \cdot [\text{Met}] \cdot P_{\text{met}} \), where \( S_{\text{ERET}}^0 \) is the transmitted ERETIC signal, \( f_r \) depends on the receiver gain and \( f_c \) depends on the loading of the receiving coil. The peak area of a metabolite is represented by \( S_{\text{met}} = f_r \cdot f_c \cdot k \cdot [\text{Met}] \cdot P_{\text{met}} \), where \( k \) does not depend on receiver gain or on receiving-coil loading, \([\text{Met}]\) is the concentration of the metabolite and \( P_{\text{met}} \) is the number of equivalent protons. The \( f_r \) factor can differ for the ERETIC signal, the water spectra or the water-suppressed spectra. However, these differences are well-known and can be taken into account.

Therefore, \( S_{\text{met}} = \frac{k \cdot S_{\text{ERET}} \cdot P_{\text{met}}}{S_{\text{ERET}}} \). Normalized areas \( I_{\text{met}} \) were then calculated as \( I_{\text{met}} = \frac{S_{\text{met}}}{S_{\text{ERET}} \cdot P_{\text{met}}} \), which were proportional to concentration \([\text{Met}]\), and independent of the sensitivity of the receiving chain and the number of equivalent protons.

Calculation of metabolite concentrations

For phantom B and the 10 volunteers, concentrations of tNAA (total N-acetylaspartate), creatine and choline were calculated in two different ways (neglecting relaxation):

(i) using the water signal as reference:

\[
[\text{Met}]_{\text{pat}} = \frac{[\text{Wa}]_{\text{pat}} \cdot S_{\text{pat}}}{S_{\text{Wa}}} \cdot P_{\text{met}} = \frac{[\text{Wa}]_{\text{pat}} \cdot I_{\text{pat}}}{I_{\text{Wa}}}
\]

(ii) using the ERETIC signal as reference:

\[
[\text{Met}]_{\text{pat}} = \frac{[\text{ChoA}]_{\text{pat}} \cdot I_{\text{pat}}\text{Cho}}{I_{\text{Cho}}}
\]

where the labels pat and A correspond to measurements on the patient (or on phantom B) and to calibration by phantom A, respectively.

Finally, in the volunteers, the concentration \([\text{Met}]\) in mmol L\(^{-1}\) was converted into \( C \), concentration expressed in mmol.kg\(^{-1}\)ww (millimoles per kilogram wet weight), by taking into account the density of brain tissue [10].

![Figure 1](image)

Sequence used to produce the ERETIC signal (A); the real part of the ERETIC signal (B) and its Fourier transform (C). \( \Delta = 5 \) ms.
Figure 2  A typical normal volunteer brain spectrum (A) and the spectrum from a phantom (B) containing NAA, creatine, myoinositol, glutamate and choline in a phosphate buffer (pH = 7.2). Black line = experimental spectrum; gray line = simulated spectrum. The x axis is the chemical shift in ppm, and the y axis is the intensity of peaks in arbitrary units.

Table 2  Concentrations ± SD (mmol.kg⁻¹ww) calculated for 10 volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>tNAA</th>
<th>Choline</th>
<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref Water</td>
<td>13.0 ± 2.2</td>
<td>12.3 ± 2.0</td>
<td>1.7 ± 0.2</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>Ref ERETIC</td>
<td>33.9 ± 1.1</td>
<td>12.3 ± 2.0</td>
<td>1.6 ± 0.2</td>
<td>6.5 ± 0.9</td>
</tr>
</tbody>
</table>

The concentrations were calculated for the five experiments. The SD measured by the ERETIC method were the same as or smaller than those measured with the water-signal reference method. Furthermore, the deviations from true values were similar with the two methods.

Results

Measurements in vitro

The accuracy and precision of the ERETIC method were determined in vitro by measuring the water and metabolite concentrations in phantom B. Phantom A was used for calibration of the reference signal. The metabolite concentrations in phantom B were also measured using the water signal as reference. The results are presented in Table 1 with the true concentrations (measured by high-resolution NMR). The means ± SD were calculated for the five experiments.

Measurements in vivo

We acquired the proton brain spectra of 10 volunteers. An ERETIC reference signal was acquired at the beginning of the examination of each volunteer. Calibration acquisition, using phantom A, was carried out at the beginning of each half-day that the volunteers were examined.
Metabolite concentrations were in good agreement with values found in the literature for normal human white matter [11]. Concentration values obtained with the ERETIC method were plotted against those obtained with the water-signal reference method (Fig. 3), and show a clear linear correlation.

Discussion

Absolute concentration quantification

In most studies in the literature of 1H-SRM in human pathology, the results are expressed in the form of relative intensities (ratio). This is a simple method, but its main drawback is that it ignores the overall variations in the metabolites, particularly of creatine, the denominator that is most frequently used. Furthermore, the use of metabolite ratios leads to significant variability in the results compared with the absolute quantification of metabolite concentrations [12].

Many strategies for absolute quantification have been applied [3]. The method involving an external reference [13] uses a vial of the reference solution — of known concentration and relaxation times — placed inside or near the coil. However, this external tube can distort the static magnetic field and disturb removal of the water signal. The principle of calibration by an external phantom (also called ‘replace-and-match’) involves replacing the patient with a phantom that simulates, as perfectly as possible, the load factor of the coil obtained in vivo. Nevertheless, implementation of this technique is not always straightforward and can be time-consuming [3]. Calibration by the internal water signal is a method widely used in the literature [3,14]. In the same voxel, acquisitions with and without removal of the water signal are carried out. The water concentration obtained without suppression is then used as an endogenous reference. It is assumed that the water concentration is essentially stable in healthy subjects and remains fairly constant in edema [15], it can vary substantially due to disease [16,17]. The principle of reciprocity assumes that the external current necessary to induce certain spatially localized B1 is inversely proportional to the current induced in the same antenna by a given B1 [18]. This calibration strategy requires determination of the local B1 field, which can be achieved by measuring the amplitude of the current necessary to obtain a maximum signal.

Nevertheless, despite the undeniable progress in this field, as yet, no method of absolute quantification has achieved consensus [3]. The ERETIC method could overcome many of the drawbacks of the other strategies [4] but, up to now, it has not been used for diagnosis because of the standard hardware configurations of clinical imagers.

ERETIC as reference

The results obtained with the ERETIC method are similar to those obtained by high-resolution NMR (for phantoms) and to the values found in the literature (for volunteers) [11]. In addition, there is close correlation between the values obtained using the ERETIC method and those found with the water signal as reference (Fig. 3). These measurements were performed in volunteers and the water-signal reference induced no biases, so it may be concluded that both methods offer accurate data. This suggests that, in patient examinations, the uncertainty of the water content will not be a source of error when the ERETIC method is used. In addition, water content can be measured as another parameter for pathology evaluation.

The constraints of using the ERETIC method are that:

- calibration acquisition has to be performed on a phantom with known concentrations;
- the ERETIC signal has to be measured at the beginning of each examination.

In this preliminary study, we performed the calibration on every half-day that examinations were carried out. However, the frequency required has yet to be scientifically evaluated, as it is likely that less frequent calibration may still provide good results. As for the ERETIC measurement itself, it takes less than 1 min, and so will not cause a significant increase in total examination time.

The ERETIC signal amplitude is only constant if the loading of the transmitting coil is the same in all experiments. According to our measurements, this was indeed the case, probably because there were no major differences in the volunteers’ morphologies. However, if the method is applied to a large number of volunteers and patients, this will no longer be true. However, this drawback can be avoided by using a receiving-only head coil. This means that RF pulses will be sent by the body coil and, because of the principle of reciprocity [18], body-coil loading variations may be quantified by measuring the transmitter voltage of a non-selective 90° pulse. This calibration has to be systematically done at the beginning of each regular examination, but it is easy to implement.

In addition, new strategies have recently been proposed [19–21] that can improve the performance of the ERETIC method with minimal hardware modification. In Hossu et al. [21], the ERETIC signal was produced by an external waveform generator and continuously sent without synchronization through a copper coil. This strategy appears to provide good stability of the ERETIC peak. However, the approach requires a greater signal for post-processing [21]. No data have been found for NMR-to-ERETIC signal ratios or for in vivo results.

The method proposed in the present study does not require additional hardware, and the sequence used to produce the signal is easy to implement using the software provided by all manufacturers. This means that implementation of this method in commercial MRI scanners will not cause technical difficulties. However, to detect the ERETIC pulse with the coil in its regularly loaded state, it is crucial to prevent detuning of the head coil during detection. This requires close cooperation with the manufacturer to avoid any material damage.

Conclusion

We have demonstrated that the ERETIC method can be used with clinical MRI scanners that have only one RF channel.
Our preliminary results, obtained in a small population of volunteers, confirm the reliability of the technique and its avoidance of many of the drawbacks of other absolute-quantification methods. However, its further evaluation is necessary in a wider population of patients within the framework of a clinical research protocol.

**Conflict of interest statement**

None.

**References**


