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Original paper

Choline-containing compounds quantification by ^{1}H NMR spectroscopy using external reference and noise measurements

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ABSTRACT

Proton magnetic resonance spectroscopy (^{1}H-MRS) is largely exploited in clinical settings to non-invasively investigate chemical compounds in human tissues. Applications of ^{1}H-MRS in oncology field are connected to the detection of abnormal levels of choline compounds in more active tumours, providing useful information for cancer diagnosis and treatment monitoring. Since benign lesions may also show presence of a choline peak, implementing absolute evaluation will help differentiating benign from malignant tumours. An external reference procedure was described to provide choline quantification in standard unit of measurements. Spectra were acquired on a 1.5 T scanner using both phantoms and healthy volunteers with a PRESS sequence. The implemented quantification procedure used metabolite and noise measurements on the spectrum to remove large part of scanner settings contributing to metabolites of interest. A standard quantification was also used to compare performances of the noise-based method. *In vitro* quantification had accuracy and precision in the range (95–99)% and (5–13)%, respectively. When applied to *in vivo* studies on healthy volunteers, the method provided very close values of choline concentration, more exactly (1.73 ± 0.24) mmol/l. The method proposed can quantify the proper choline content in phantoms as well as in human structures, as brain. The method is ease of use, computational costless and it can be rapidly calibrated and implemented in any centre.

Introduction

In vivo localized proton single-voxel magnetic resonance spectroscopy (^{1}H-MRS) is an application of magnetic resonance that non-invasively provides chemical information about metabolite content in human tissues [1]. The first clinical usage of ^{1}H-MRS came in the 1980s and since then ^{1}H-MRS has become an important application in oncology to study patients with brain cancer [2].

Recently, ^{1}H-MRS acquisitions have been expanded to cancer investigation in other regions of the body including prostate [3–7], breast [1,8–12] and musculoskeletal lesions [13–15]. The potential value of ^{1}H-MRS in oncology is usually connected to the detection of abnormal levels of choline compounds, typically found in more active tumour masses [16,17]. Such molecular information is expected to be useful for cancer diagnosis, treatment monitoring and patient follow up.

However, some recent studies demonstrated that choline signals could also be detected in benign lesions and in normal tissues [10,12]. For this reason a quantitative assessment is required to accurately establish choline levels and, thus, distinguish between the different pathologies.

Absolute determination of metabolite concentrations by ^{1}H-MRS has been attempted by either internal or external standard references and using known concentrations [18]. The ratio of the area under a metabolite peak to the area of internal reference is commonly used as a surrogate of metabolite concentration since it is very easy to compute [19–22]. Absolute quantification (AQ) by external reference offers some advantages: higher reproducibility of results can be achieved, concentrations can be expressed in mM units, internal reference, such as water or creatine (for brain tumour masses), becomes unnecessary and tracking metabolite variations would be more representative of actual clinical modifications during longitudinal studies. The drawbacks of external reference AQ include time consuming data acquisition (i.e. phantom calibrations) and more extensive data analysis [23].

This work describes an external reference quantification procedure to account for scanner contribution to the signal in order to evaluate choline concentrations in human tissues. The method is based on the observation that scanner settings simultaneously influence signals generated by metabolites as well as thermal noise.

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Hence, metabolite concentrations, independent of scanner technical settings, can be obtained using the ratio between the metabolite signal and spectrum noise content.

Materials and methods

Acquisition protocol

Phantom studies and *in vivo* measurements were performed on a clinical whole-body scanner (Signa HDx, GE Medical System, Milwaukee) operating at 1.5 T with standard ^{1}H-MRS acquisition software provided by the manufacturer. A body coil was used for signal transmission and a dedicated eight channel high resolution head coil (8HRBRAIN, MRI Device Corporation, Wisconsin) as a receiver for both MR imaging and ^{1}H-MRS. A series of T2-weighted fast gradient echo (FGRE) images were acquired in the three spatial planes to correctly place the spectroscopic voxel. Spectra were collected with a point-resolved spectroscopy sequence (PRESS) [24] using the following acquisition parameters: repetition time (TR) 1500 ms, echo time (TE) 35 ms [25], NEX = 8, total number of scans = 128, spectral width = 2500 Hz, sampling points = 2048.

A short TR was selected to maintain scan duration unchanged for both phantom studies and *in vivo* acquisitions. In fact, increasing scan time in clinical settings would decrease patient compliance, resulting in motion artifacts and would reduce overall patient throughput. Furthermore, a longer TR would reduce the total number of acquisitions, yielding a lower signal to noise ratio (SNR).

Automatic shimming on the unsuppressed water signal ensured that full width at half maximum (FWHM) was equal to or less than 5 Hz. If this condition was not met automatically, manual shimming was performed to adjust the FWHM to a lower value. Total imaging time, including pre-imaging shimming adjustments and water suppression, was less than 5 min.

Phantoms

In vitro metabolite quantification was carried out on a phantom provided by the scanner manufacturer and on several home-built phantoms. The first was a sphere filled with an aqueous solution of biochemicals typically present in brain tissue. The home-built phantoms were two sets of vials: the first was designed as a training set for the AQ of choline and consisted of five Falcon tubes (each one with a volume of 50 ml), filled with a choline chloride (Sigma–Aldrich, Steinheim, Germany) and bi-distillate water solution with concentrations ranging between 0.5 and 4 mmol/l. The second, referred to as a validation set, consisted of six vials in which choline concentrations were different with respect to the first set and ranged between 0.75 and 2.25 mmol/l. All of the aqueous solutions of choline chloride were prepared in tubes of 10 cm in length and 3 cm in diameter at room temperature.

During acquisitions each sample was kept in a water container to optimize field homogeneity, reducing magnetic susceptibility differences at vial–air interfaces. Coil resistive load was increased to simulate body presence by posing a $15 \times 15 \times 40$ cm^3 box filled with NaCl and $CuSO_4 \bullet 5H_2O$ solution beside the choline-containing Falcon tube, so that measurements were more representative of *in vivo* studies.

In vitro measurements

A pre-scan procedure was run before each acquisition to define optimal settings for analogue/digital receiver gains, transmitter gains and water centre frequency. In fact, radiofrequency excitation caused sample temperature rising and consequent metabolite frequency shifts which, if neglected, might have produced errors during spectra acquisitions. If the pre-scan process had been omitted, the default frequency as set by the scanner for excitation would not have been optimal for the prescribed scan, thus the metabolite content in the examined voxel would not have been correctly stimulated. Before using the phantoms for calibration, the influence of several scanning factors on signal amplitude was investigated: a large quantity of spectra were acquired on the manufacturer's phantom to evaluate signal variations correlated to analogue gain (G1), digital gain (G2) and voxel size (V). Measurements were taken by changing one parameter at a time and holding the others at a constant value. For example, maintaining fixed gain values (G1 and G2) while changing voxel size ensured that all signal variations were ascribable to the different voxel sizes.

Data processing

At the end of the acquisition process, raw data were transferred from the scanner to a dedicated workstation where post-processing took place. Spectra analysis was performed by homemade tools and the reconstruction process consisted of the following steps: phase correction, apodization, zero-filling, Fourier transform and baseline correction. In particular, the parameter to implement zero order phase (φ) correction was obtained by Bolan's approach [12]. Phase was measured and corrected using the average of two different autophasing methods: 1) the phase of the first 100 time domain points was fitted to a straight line to obtain φ at time $t = 0$ and 2) φ was chosen to maximize the smallest value of the real part of the spectrum. A robust estimate of φ was produced using the average of φ found when applying these two methods.

Apodization was carried out using a 1.5 Hz line broadening decaying exponential function, while zero-filling procedure doubled points in the spectrum, increasing the frequency point number. Following these preliminary corrections, water suppressed/unsuppressed FIDs were Fourier transformed and the resulting spectra were frequency referenced by setting the maximum water peak to 4.7 ppm. The procedure was applied to all FIDs acquired by each individual head coil channel.

The final spectrum was constructed from the eight components, each weighted by its SNR [8]. Water peak linewidth was used as the parameter to accept or reject a spectrum before post-processing steps; in this study the water peak linewidth threshold was set to 3 Hz and this value was meant only for *in vitro* studies. Baseline correction was finally applied to the resulting spectrum as shown in Heuer's work [26]: to decide whether the i-th point lay on baseline, a rectangular 15 spectral points width window was centred on it. Maximum and minimum values within this window were extracted in order to compare their difference to noise standard deviation. The i-th point was classified as baseline if the absolute difference between minimum and maximum values did not exceed twice the noise standard deviation. The final baseline was constructed by spectrum fragments connected with straight lines.

The following analysis was implemented to automatically detect the choline peak and to measure its area. Detection algorithm was based on the analysis of first and second spectrum derivatives. The first derivative was computed in the range $(-1, 4.5)$ ppm; then the first derivative zero crossing points were determined and, where each zero crossing point was found, the second derivative magnitude was compared to a threshold value to avoid spurious peak detection. Peaks found in this step were compared to an adaptive value, i.e. if the peak to noise ratio was greater than three, then the algorithm returned the value in ppm where the peak was detected. Once identified, it was fitted to a Gaussian bell using nonlinear least-square regression. The fitting procedure was included in the post-processing step to remove possible spurious signals superimposed on choline tails. Once the fitting procedure was

completed, the peak area (A_{raw}) was measured. This was the starting point for the quantification procedure.

AQ procedure

A quantification method was implemented in this work to offset deep and time consuming parameterizations of MR scanners, typical of conventional methods. The rationale of the procedure was based on considerations regarding acquisitions on the sphere manufacturer phantom and the evaluation of signal variations correlated to scanner parameters. In fact, scanner settings (analogue and digital gains) affect both signals generated by metabolites as well as signals due to thermal noise [27,28], as depicted in Fig. 1. The ratio between the area beneath choline peak to the area of a selected spectral region far from the metabolites present in the specimen is not influenced by the scanner settings, thus making it possible to obtain a choline area due principally to the actual concentration of metabolite. As sketched in Fig. 2, noise was measured in the flat baseline region (>8 ppm) of each spectrum, where no metabolites were present [29,30]. The noise integration interval to compute its area was set to 100 Hz, in order to acquire a representative number of points describing noise spectrum content. Equation (1) represents the pivotal key of the procedure here proposed,

$$A_{Cho} = \frac{A_{raw}}{A_{noise}} \quad (1)$$

where A_{raw} is the raw area measured under the peak of choline with no corrections for scanner settings, A_{noise} is the area under noise spectrum and A_{Cho} is the desired area attributable to choline concentration with no scanner setting dependence. To define noise integration interval we computed the mean area under the flat region of the spectrum where no metabolites could be detected. Mean area under noise was defined as the area under the noise divided by the number of points belonging to the integration interval or, equivalently, the interval length in Hz (or ppm) units. The mean area computed under noise reached a constant value in a 100 Hz interval, and was no longer affected by strong oscillations characterizing the very first points of this function.

Furthermore, A_{Cho} should still be corrected for longitudinal and transversal magnetization relaxation phenomena and temperature; nevertheless, in neglecting these corrections the procedure would systematically underestimate the A_{Cho} measurement. In case of *in vitro* experiments, such systematic errors vanished when *in vitro* area A_{raw} was converted to mM concentration units. In case of *in vivo* measurements, such correction was still neglected in view of various results available in literature [31,32].

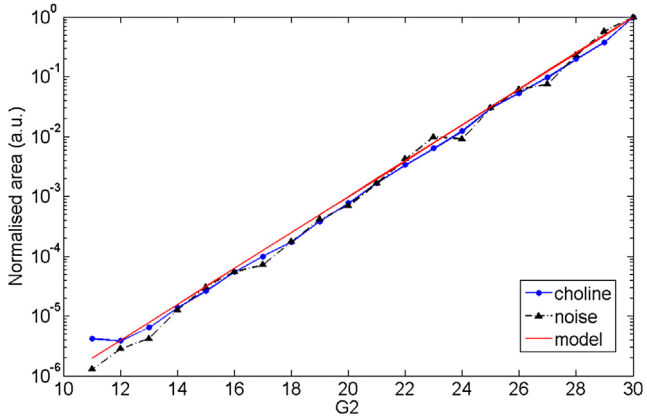

Figure 1. Noise and choline peak areas at different G2 settings. Results are compared with a scanner model describing the theoretical signal variation with gain. The noise-based quantification method removes signal variation due to scanner settings since noise and choline areas have similar behaviour when technical parameters change.

The quantity A_{Cho} was still a surrogate of choline levels; in fact, a calibration factor was needed in order to change A_{Cho} to [Cho]. The calibration factor was obtained by exploiting the connection between A_{Cho} and well-known contents of choline of the training dataset phantoms and acquiring each available concentration at different voxel sizes (5.8, 3.4, 1.7 and 0.7 cm^3). Each experimental point was obtained as the mean value calculated on three consecutive acquisitions. Using the mean calibration curve computed on the 4 lines shown in Fig. 3, a calibration curve emerged whose slope was (4.23 ± 0.14) l/mmol with $R^2 = 0.98$.

Then for each concentration belonging to the validation set, 3 measurements were performed for each voxel size to compute the mean concentration (μ) and the corresponding standard deviation (σ).

The results of this new methodology were compared with a standard quantification method using the conventional phantom replacement technique [18,33].

Statistical analysis

The capability of the proposed procedure to finely quantify correct choline concentration was validated on a second set of vials. Accuracy (Equation (2)) and precision (Equation (3)) of the method were reported as parameters that summarize the goodness of the quantification tool.

$$a = \left(1 - \left|\frac{\text{measured} - \text{expected}}{\text{expected}}\right|\right) \cdot 100 \quad (2)$$

$$p = \frac{SD_{measured}}{\text{measured}} \cdot 100 \quad (3)$$

where measured, expected and $SD_{measured}$ stand for average, true and standard deviation values of several measurements performed on any individual vial, respectively.

Stability of the scanner and measurement reproducibility were verified on a phantom experimental setup. Reproducibility refers to the precision of the measurement and the ability to repeat and reproduce the experiment [33]. In this study, reproducibility is interpreted as measurements with phantom repositioning and reshimming. In the sphere phantom twenty-seven measurements were taken in the same voxel (volume = 18 × 18 × 18 mm^3) with repositioning and reshimming procedure. The voxel was placed in the centre of the phantom and external temperature was verified regularly from the temperature strip indicator on the phantom.

In vivo quantification

At the end of this study the quantification procedure was applied to *in vivo* acquisitions. Measurements were performed on 20 healthy volunteers (9 men and 11 women), between 19 and 50 years of age (mean ± std = 29.35 ± 7.41 years). The volume of interest was isotropic, with a side length of 20 mm and it was placed in parietal white matter for all subjects. Particular care was taken to optimize magnetic field homogeneity and water suppression by localized adjustments. The same acquisition sequence was set for *in vitro* and *in vivo* experiments and total scan time was approximately 5 min per subject.

Data acquired on healthy volunteers were elaborated according to the scheme described for *in vitro* studies. Such measurements were aimed at assessing choline distribution in brain tissues of

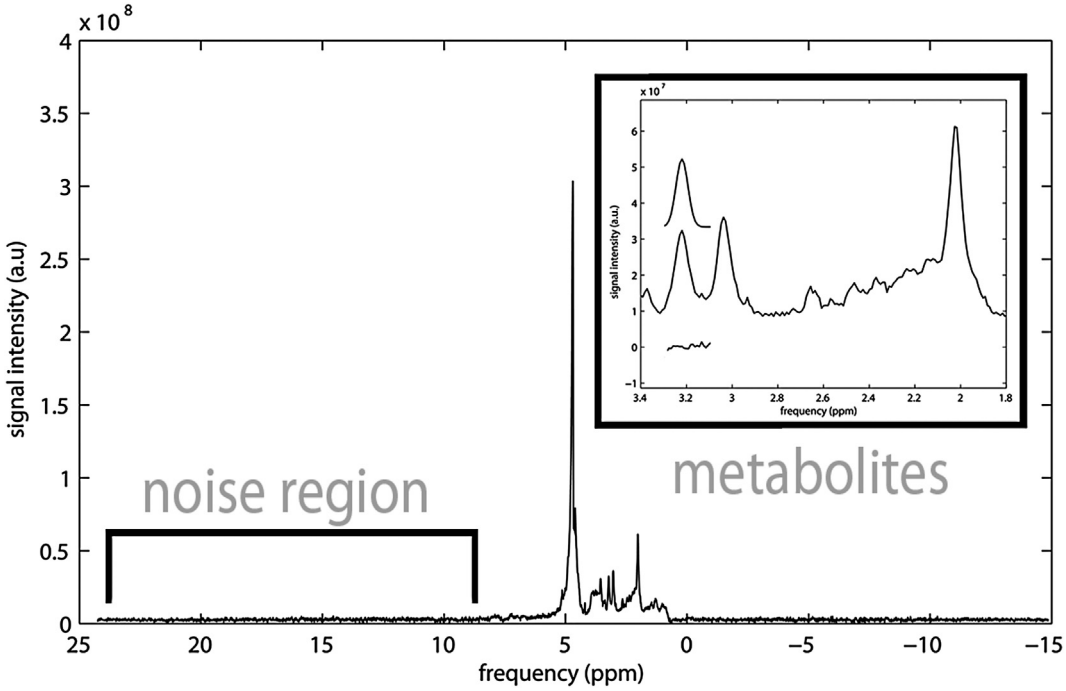

Figure 2. Example of *in vivo* acquisition showing the complete frequency range in ppm with metabolites and noisy region. The small window depicts the spectrum zoom in the range (1.8–3.4) ppm with main brain metabolites. The model fit for the choline peak is shown above the spectrum and residuals are shown underneath.

selected subjects. The results were then compared to published data available in literature [22,31,32,34].

Results

In vitro study

Table 1 shows validation set choline concentrations obtained by exploiting the noise-based method and a standard quantification procedure. Agreement between concentrations measured by the described procedure and the choline actually present in samples was evaluated: accuracy and precision were in the range of (95–99)% and (5–13)%, respectively.

As regards measurement reproducibility, a little variability was found during this study. The standard method showed the worst performances; in fact, differences between reference and estimated values were up to 17.0%; on the contrary, the method here proposed demonstrated more stable performances; maximum variation was 5.2%.

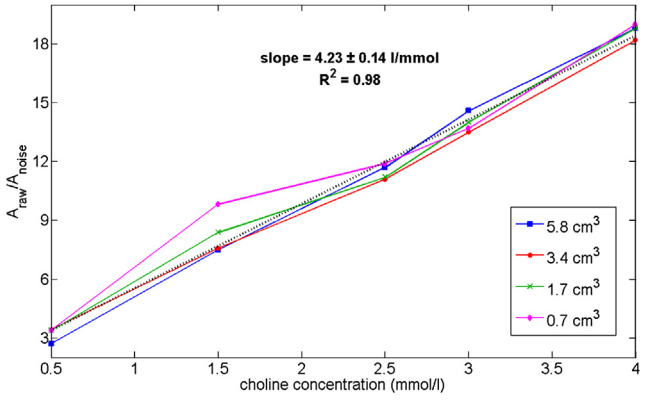

Figure 3. Example of several calibration curves obtained from the training set at different voxel sizes. It is important to note how experimental points disperse at a constant choline concentration when different volumes of analysis are investigated. The four lines are basically superimposed, meaning that no difference can be observed in the ratio A_{raw}/A_{noise} if voxel size is changed. The dotted line represents the interpolation of the mean curve obtained from the four experimental lines reported.

In vivo study

Table 2 and Fig. 4 summarize *in vivo* quantification results carried out on 20 healthy volunteers. The method proposed measured the same amount of choline as a standard quantification procedure did in brain parietal white matter, but distribution of the results differed according to patient variability.

Discussion

Among molecular imaging techniques, ^{1}H-MRS had gained large success as a viable tool to analyse metabolite content in tissues, opening a new way to characterize lesions detected by conventional MRI studies [35]. One advantage of ^{1}H-MRS is its ability to measure the concentration of biochemicals in regions of interest. AQ is usually performed by dedicated software to process spectroscopic data and to extract absolute concentrations of metabolites. Two of these are particularly worth mentioning, the

Table 1
Validation measurements on concentrations belonging to the validation set to test goodness of quantification. Results obtained with a standard method of quantification are also reported.

Reference choline concentration (mmol/l)	Noise-based method ($\mu \pm \sigma$) mmol/l	Standard method ($\mu \pm \sigma$) mmol/l
2.25	2.29 ± 0.07	2.28 ± 0.07
2.00	1.99 ± 0.25	2.09 ± 0.19
1.75	1.82 ± 0.14	1.72 ± 0.12
1.25	1.23 ± 0.08	1.22 ± 0.07
1.00	0.94 ± 0.13	0.91 ± 0.13
0.75	0.73 ± 0.07	0.76 ± 0.06

Table 2
In vivo measurements of brain choline content in parietal white matter for 20 healthy volunteers. Choline levels were measured using the noise-based procedure described in this study and a conventional quantification method for comparison.

	Noise-based method (mmol/l)	Standard method (mmol/l)
$(\mu \pm \sigma)$	(1.73 ± 0.24)	(1.73 ± 0.20)
Confidence interval (95%)	(1.63–1.84)	(1.65–1.82)

LCModel and the jMRUI [36]. The software designed to perform AQ analysis typically measures the area beneath a metabolite peak, then by a proper calibration factor this area can be converted into concentration units. Two main procedures allow for calibration which use an internal or external reference.

Using internal reference, chosen by a large number of research groups [17], has several advantages with respect to using external reference; in fact, separate calibration experiments as well as partial volume corrections are unnecessary. However, such a procedure may increase acquisition time to allow for water T_1, T_2 and proton density measuring [1,37,38].

When AQ is meant to monitor treatment efficacy, it is necessary to have a very stable reference to ascribe observed metabolite concentration changes to a baseline to highlight the concrete metabolite behaviour, while maintaining reproducibility of voxel localization in the same region of analysis.

This work describes a method for AQ of choline levels by ^{1}H-MRS studies. It uses an external reference to convert the area beneath choline peak to metabolite concentration. The choice of an external reference was made in light of studies which demonstrated that internal reference concentration might vary [17,39], contrary to the assumption that their concentrations did not change when pathological conditions changed [1]. Methods based on external references may normally suffer from the partial volume effect, thus requiring correction strategies to account for it. Nevertheless, in this study this potential improvement was not considered because there is absolutely no compartmentation in vials or in the manufacturer phantom. When considering healthy volunteers, we chose homogeneous regions greater than 3.5 cc in volume to get appropriate SNR in the spectrum. In any case, a correction for partial volume is still possible when the procedure is translated to *in vivo* studies, in the case of a mixture of different tissue types within a given voxel [40].

Furthermore, temperature effects on signal intensity should be considered when performing *in vivo* quantification. As noted by Reyngoudt et al. neglecting temperature would result in ~5% error in concentrations [33].

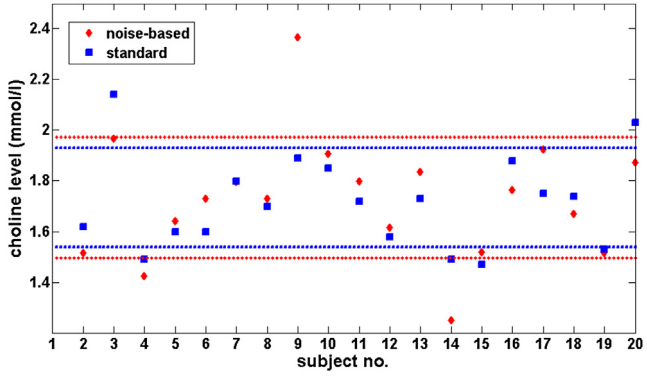

Figure 4. Results of the two implemented methods for quantification of brain choline levels in 20 healthy volunteers. Dotted lines represent the interval $(\mu \pm \sigma)$ for each dataset.

With different relaxation times between metabolites and reference compound, the acquired signal intensity of each resonance must also be corrected [41]. This can be achieved using correction factors, but they require knowledge of T_1 and T_2 relaxation times in tissues. When performing acquisitions at short echo times, T_2 effects cause small corrections, about 3%, that can be ignored to avoid time consuming determination of T_2 values [42,43]. Evaluation of T_1 relaxation times is often impractical in a clinical setting, due to the excessive examination time needed to measure it and its minor contribution to data quantification [42]. However, the correction is still possible when the operator can estimate T_1 relaxation times.

The procedure developed in this study was employed to detect and measure choline levels in human tissues, but its main application is actually the study and evaluation of tumour masses. *In vivo* ^{1}H-MRS can detect a resonance at 3.2 ppm, containing contributions from several different compounds, primarily glycerophosphocholine, phosphocholine and choline, including other metabolites such as glycerophosphoethanolamine, phosphoethanolamine, betaine, myo-inositol and taurine [41,44]. At lower field strengths used for *in vivo* work (1.5–4 T), this complex mixture of heavily overlapping resonances cannot be spectrally resolved and, thus, appears as a single peak, termed total choline (tCho) [12]. The levels of metabolite measured *in vivo* are then overestimated, but only slightly as choline signal intensity corresponds to 9 protons ($N(CH_3)_3$ resonance), whereas the signal intensity of all the other compounds corresponds to 1 or 2 protons (CH or CH_2 group), except for betaine whose signal also corresponds to 9 protons.

Elevated choline levels are normally observed in neoplastic tissues, thus, more accurate diagnosis would be possible if choline levels are measured in such tissues [45,46]. The development of a simple calibration procedure like the one used in this study can facilitate the implementation of AQ in clinical routine. Initially, it was thought that the procedure describing signal changes related to scanner settings, voxel size and physical phenomena was the best way to make choline quantification independent of MR scanners. However, empirical measurements have indicated that the method here developed can ensure precision and accuracy indices very close to those attainable with standard and more complicated AQ techniques, while greatly simplifying execution.

In addition, reproducibility tests reveal that the quantification procedure was less sensitive than a conventional method to scanner drifts, hardware and software upgrades. In fact, the scanner underwent several software improvements and hardware checks regularly throughout this study. Nevertheless, accuracy was found to be highly stable with respect to these adjustments because noise as well as metabolite signals have similar perturbations soon after periodic routine inspection and scanner maintenance. According to Equation (1), perturbations appear simultaneously at the numerator and denominator, thus mutually compensating their effects.

The method's ease of calibration is another strong point: it is possible to calibrate the procedure using a unique vial filled with a well-known concentration of metabolite. Implementation of the AQ technique in other facilities is simpler than methods which require a full recalibration process like the LCModel [36]. Furthermore, the procedure developed maintained acquisition times unchanged with respect to usual clinical settings: all parameters needed for quantification purposes were obtained from traditional spectroscopic sequences without increasing acquisition times.

Translation of the method described in this work to *in vivo* studies looks promising. In fact, choline levels in parietal white matter measured by AQ were quite close to the results obtained in similar studies by Michaelis and Pouwelset [31,32]. Both these authors carried out measurements on the same brain region we chose and they found that choline concentration was

(1.8 ± 0.3) mmol/l and (1.68 ± 0.27) mmol/l, respectively. In fact, we chose to cite these two authors because the relative error they achieved was highly compatible with the one obtained in this work. Other studies showed even worse relative errors [22,34].

The current methodology can be extended to the quantification of other metabolites in brain and other organs, but care must be paid to calibrate areas by means of an appropriate phantom. Heinzer-Schweizer et al. [47] have recently demonstrated that the ERETIC technique is a convenient method for accurate and reproducible absolute quantification (in mmol/l) of *in vivo* metabolites in humans. They used a synthesized RF pulse to produce an additional peak in NMR spectrum and, after calibration of the electronic signal, the method allowed signal quantification of all metabolites. However, its successful application depends on the use of a homogeneous volume coil and B_1 mapping and correction. In addition, the ERETIC method is susceptible to errors in power optimization.

Since both spectral and spatial resolutions are linearly related to the strength of the applied magnetic field, the use of scanners operating at higher magnetic field strengths (3 T or higher) would be valuable to achieve better quantification results [48]. Comparing the results between 1.5 T and 3 T scanners, Kim et al. [49] found a 55% SNR improvement at short TE but only a 4% SNR improvement at intermediate TE; Barker et al. [50] used an STEAM sequence to evaluate spectroscopy results in human brain tumours and found a 28% SNR improvement at short TE when acquisition was performed on 3 T magnetic field instead of 1.5 T.

Conclusions

In this study metabolite concentrations in parietal white matter of twenty health volunteers were measured by a point-resolved ^{1}H-MRS sequence. AQ was achieved by calibration of spectral evaluations with data from metabolite model solutions measured under identical experimental conditions. The aim was to implement a procedure to correct acquisitions from scanner settings contribution in order to have a spectrum independent of acquisition parameters (gains and volume of interest).

In conclusion, *in vivo* choline concentrations were in good agreement with those determined by other methods in literature. The proposed method is simple and can readily be applied in any MR centre without the need for complicated corrections or time consuming calibration procedures.

References

[1] Baik HM, Su MY, Yu H, Mehta R, Nalcioglu O. Quantification of choline-containing compounds in malignant breast tumors by ^{1}H MR spectroscopy using water as an internal reference at 1.5 T. Magma 2006;19:96–104.

[2] Shah N, Sattar A, Benanti M, Hollander S, Cheuck L. Magnetic resonance spectroscopy as an imaging tool for cancer: a review of the literature. J Am Osteopath Assoc 2006;106:23–7.

[3] Yacoe ME, Sommer G, Peehl D. In vitro proton spectroscopy of normal and abnormal prostate. Magn Reson Med 1991;19:429–38.

[4] Schick F, Bongers H, Kurz S, Jung WI, Pfeffer M, Lutz O. Localized proton MR spectroscopy of citrate in vitro and of the human prostate in vivo at 1.5 T. Magn Reson Med 1993;29:38–43.

[5] Kurhanewicz J, Vigneron DB, Hricak H, Narayan P, Carroll P, Nelson SJ. Three-dimensional H-1 MR spectroscopic imaging of the in situ human prostate with high (0.24–0.7-cm^3) spatial resolution. Radiology 1996;198:795–805.

[6] Swindle P, McCredie S, Russell P, Himmelreich U, Khadra M, Lean C, et al. Pathologic characterization of human prostate tissue with proton MR spectroscopy. Radiology 2003;228:144–51.

[7] Cheng LL, Burns MA, Taylor JL, He W, Halpern EF, McDougal WS, et al. Metabolic characterization of human prostate cancer with tissue magnetic resonance spectroscopy. Cancer Res 2005;65:3030–4.

[8] Roebuck JR, Cecil KM, Schnall MD, Lenkinski RE. Human breast lesions: characterization with proton MR spectroscopy. Radiology 1998;209:269–75.

[9] Kvistad KA, Bakken IJ, Gribbestad IS, Ehrnholm B, Lundgren S, Fjøsne HE, et al. Characterization of neoplastic and normal human breast tissues with in vivo (1)H MR spectroscopy. J Magn Reson Imaging 1999;10:159–64.

[10] Jacobs MA, Barker PB, Bottomley PA, Bhujwalla Z, Bluemke DB. Proton magnetic resonance spectroscopic imaging of human breast cancer: a preliminary study. J Magn Reson Imaging 2004;19:68–75.

[11] Meisamy S, Bolan PJ, Baker EH, Bliss RL, Gulbahce E, Everson LI, et al. Neoadjuvant chemotherapy of locally advanced breast cancer: predicting response with in vivo ^{1}H MR spectroscopy – a pilot study at 4 T. Radiology 2004;233:424–31.

[12] Bolan PJ, Meisamy S, Baker EH, Lin J, Emory T, Nelson M, et al. In vivo quantification of choline compounds in the breast with ^{1}H MR spectroscopy. Magn Reson Med 2003;50:1134–43.

[13] Fayad LM, Salibi N, Wang X, Machado AJ, Jacobs MA, Bluemke DA, et al. Quantification of muscle choline concentrations by proton MR spectroscopy at 3 T: technical feasibility. AJR Am J Roentgenol 2010;194:W73–9.

[14] Wang CK, Li CW, Hsieh TJ, Chien SH, Liu GC, Tsai KB. Characterization of bone and soft-tissue tumors with in vivo ^{1}H MR spectroscopy: initial results. Radiology 2004;232:599–605.

[15] Russo F, Mazzetti S, Grignani G, De Rosa G, Aglietta M, Anselmetti GC, et al. In vivo characterisation of soft tissue tumours by 1.5-T proton MR spectroscopy. Eur Radiol 2012;22:1131–9.

[16] Tozaki M, Fukuma E. ^{1}H MR spectroscopy and diffusion-weighted imaging of the breast: are they useful tools for characterizing breast lesions before biopsy? AJR Am J Roentgenol 2009;193:840–9.

[17] Gillies JR, Morse DL. In vivo magnetic resonance spectroscopy in cancer. Annu Rev Biomed Eng 2005;7:287–326.

[18] Jansen JF, Backes WH, Nicolay K, Kooi ME. ^{1}H-MR spectroscopy of the brain: absolute quantification of metabolites. Radiology 2006;240:318–32.

[19] Barker PB, Soher BJ, Blackband SJ, Chatham JC, Mathews VP, Bryan RN. Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. NMR Biomed 1993;6:89–94.

[20] Alger JR, Symko SC, Bizzi A, Posse S, DesPres DJ, Armstrong MR. Absolute quantitation of short TE brain 1H-MR spectra and spectroscopic imaging data. J Comput Assist Tomogr 1993;17:191–9.

[21] Kreis R, Ernst T, Ross B. Absolute quantitation of water and metabolites in the human brain II. Metabolite concentrations. J Magn Reson Ser B 1993;102:9–19.

[22] Soher BJ, Hurd RE, Sailasuta N, Barker PB. Quantitation of automated single-voxel proton MRS using cerebral water as an internal reference. Magn Reson Med 1996;36:335–9.

[23] Bakken IJ, Gribbestad IS, Singstad TE, Kvistad KA. External standard method for the in vivo quantification of choline-containing compounds in breast tumors by proton MR spectroscopy at 1.5 Tesla. Magn Reson Med 2001;46:189–92.

[24] Bottomley PA. Spatial localization in NMR spectroscopy in vivo. Ann N Y Acad Sci 1987;508:333–48.

[25] Fayed N, Olmos S, Morales H, Modrego PJ. Physical basis of magnetic resonance spectroscopy and its application to central nervous system diseases. Am J Applied Sci 2006;3:1836–45.

[26] Heuer A, Haeberlen U. A new method for suppressing baseline distortions in FT NMR. J Magn Reson 1989;85:79–94.

[27] Hoult DI, Richards RE. The signal-to-noise ratio of the nuclear magnetic resonance experiment. J Magn Reson 1976;24:71–85.

[28] Redpath TW. Signal-to-noise ratio in MRI. Br J Radiol 1998;71:704–7.

[29] Baek HM, Chen JH, Yu HJ, Mehta R, Nalcioglu O, Su MY. Detection of choline signal in human breast lesions with chemical-shift imaging. J Magn Reson Imaging 2008;27:1114–21.

[30] Huang W, Fisher PR, Dulaimy K, Tudorica LA, O'Hea B, Button TM. Detection of breast malignancy: diagnostic MR protocol for improved specificity. Radiology 2004;232:585–91.

[31] Michaelis T, Merboldt KD, Bruhn H, Hänicke W, Frahm J. Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. Radiology 1993;187:219–27.

[32] Pouwels PJ, Frahm J. Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med 1998;39:53–60.

[33] Reyngoudt H, De Deene Y, Descamps B, Paemeleire K, Achten E. ^{1}H-MRS of brain metabolites in migraine without aura: absolute quantification using the phantom replacement technique. Magn Reson Mater Phy 2010;23:227–41.

[34] Duc CO, Weber OM, Trabesinger AH, Meier D, Boesiger P. Quantitative ^{1}H MRS of the human brain in vivo based on the stimulation phantom calibration strategy. Magn Reson Med 1998;39:491–6.

[35] Dzik-Jurasz AS. Molecular imaging in oncology. Cancer Imaging 2004;4:162–73.

[36] Helms G. The principles of quantification applied to in vivo proton MR spectroscopy. Eur J Radiol 2008;67:218–29.

[37] Bajzik G, Auer T, Bogner P, Aradi M, Kotek G, Repa I, et al. Quantitative brain proton MR spectroscopy based on measurement of the relaxation time T1 of water. J Magn Reson Imaging 2008;28:34–8.

[38] Tong ZY, Toshiaki Y, Wang YJ. Proton magnetic resonance spectroscopy of normal human brain and glioma: a quantitative in vivo study. Chin Med J 2005;118:1251–7.

[39] Li BS, Wang H, Gonen O. Metabolite ratios to assumed stable creatine level may confound the quantification of proton brain MR spectroscopy. Magn Reson Imaging 2003;21:923–8.

[40] Su Y, Thakur SB, Karimi S, Du S, Sajda P, Huang W, et al. Spectrum separation resolves partial-volume effect of MRSI as demonstrated on brain tumor scans. NMR Biomed 2008;21:1030–42.

[41] De Graaf RA. In vivo NMR spectroscopy: principles and techniques. 2nd ed. West Sussex, England: Wiley; 2007.
[42] Isobe T, Matsumura A, Anno I, Yoshizawa T, Nagatomo Y, Itai Y, et al. Quantification of cerebral metabolites in glioma patients with proton MR spectroscopy using T2 relaxation time correction. Magn Reson Imaging 2002;20: 343–9.
[43] Kim JH, Chang KH, Na DG, Song IC, Kwon BJ, Han MH, et al. 3T ^{1}H-MR spectroscopy in grading of cerebral gliomas: comparison of short and intermediate echo time sequences. AJNR Am J Neuroradiol 2006;27:1412–8.
[44] Swindle P, Ramadan S, Stanwell P, McCredie S, Russell P, Mountford C. Proton magnetic resonance spectroscopy of the central, transition and peripheral zones of the prostate: assignments and correlation with histopathology. Magn Reson Mater Phy 2008;21:423–34.
[45] Morse DL, Carroll D, Day S, Gray H, Sadarangani P, Murthi S, et al. Characterization of breast cancers and therapy response by MRS and quantitative gene expression profiling in the choline pathway. NMR Biomed 2009;22: 114–27.
[46] Fayad LM, Barker PB, Jacobs MA, Eng J, Weber KL, Kulesza P, et al. Characterization of musculoskeletal lesions on 3-T proton MR spectroscopy. AJR Am J Roentgenol 2007;188:1513–20.
[47] Heinzer-Schweizer S, Zanche N, Pavan M, Mens G, Sturzenegger U, Henning A, et al. *In-vivo* assessment of tissue metabolite levels using ^{1}H MRS and the Electric REference To access *In vivo* Concentrations (ERETIC) method. NMR Biomed 2010;23:406–13.
[48] Machann J, Schlemmer HP, Schick F. Technical challenges and opportunities of whole-body magnetic resonance imaging at 3 T. Phys Med 2008;24:63–70.
[49] Kim JH, Chang KH, Na DG, Song IC, Kim SJ, Kwon BJ, et al. Comparison of 1.5T and 3T ^{1}H MR spectroscopy for human brain tumors. Korean J Radiol 2006;7:156–61.
[50] Barker PB, Hearshen DO, Boska MD. Single-voxel proton MRS of the human brain at 1.5T and 3.0T. Magn Reson Med 2001;45:765–9.