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RESEARCH ARTICLE

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Quantification of choline-containing compounds in malignant breast tumors by ^{1}H MR spectroscopy using water as an internal reference at 1.5 T

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Abstract The quantification of choline-containing compounds (Cho) in breast tumors by proton MR spectroscopy (^{1}H-MRS) has been of great interest because such compounds have been linked to malignancy. In this study, an internal reference method for the absolute quantification of Cho metabolite in malignant breast tumors was presented using a clinical 1.5 T scanner. We performed in vitro measurements to examine the accuracy of absolute quantification using four phantoms of known choline chloride concentrations. There was a high correlation between the calculated concentrations by MRS and the known concentrations ($r^2 > 0.98$). We applied the technique to in vivo breast study conducted on 45 patients with biopsy-confirmed breast cancer. After T_1 and T_2 relaxation times were corrected, the Cho levels in this work had a range of 0.76 – 21.20 mmol/kg from 34 MR spectra of 32 patients with malignant breast lesions. This result was rather consistent with the previously published value (i.e., 1.38 – 10 mmol/kg, Bolan et al. in Magn Reson Med 50:1134–1143, 2003). Therefore, we conclude that the internal method using the fully relaxed water as a reference could be used for quantifying Cho metabolite accurately in breast cancer patients using a clinical 1.5 T scanner.

Keywords Choline · Breast cancer · Proton magnetic resonance spectroscopy · Quantification

Introduction

In vivo localized proton single-voxel MR spectroscopy (^{1}H-MRS) is a non-invasive technique that provides information on tumor metabolism, which may be used in tumor diagnosis and evaluating the therapeutic response of the tumor. Recently, ^{1}H-MRS has been increasingly applied for the detection and monitoring of breast cancer. Several studies have shown that total choline-containing compounds (Cho) may be detected in malignant breast tumors using ^{1}H-MRS techniques [1–6]. However, in some of the recent studies Cho signals were also detected in benign lesions and in normal breast tissues [7, 8]. Thus, a quantitative measurement is required to quantify the accurate levels of Cho for differentiating between different pathologies.

In some of the previous ^{1}H-MRS studies, in vivo absolute quantitation of Cho peak has been performed using an external reference with known concentrations [1, 9]. Although these studies were limited to small patient groups, they demonstrated the feasibility of quantitative ^{1}H-MRS for measuring the levels of Cho metabolite in malignant breast lesions. However, further investigations are required to determine whether the absolute Cho values reported are reasonable and reproducible.

Recently, another group applied an internal reference method to quantify Cho levels in the breast using

^{1}H-MRS at high field (4.0 T) [7]. These authors suggested in that work that internal method using water as a reference peak could be used to diagnose breast lesions and monitor response to cancer treatments. This method automatically compensates for the partial volume of adipose tissue in the voxel and leads to a molal (mmol/kg) concentration for water-soluble metabolites, whereas an external reference method does require the correction for partial volume effect and separate calibration experiments. For this reason an internal reference method was chosen in this study. To our knowledge, the present work is the first study applying an internal reference method to investigate the absolute quantification of Cho levels in malignant breast tumors using ^{1}H-MRS at 1.5 T. A quantitative measurement is expected to provide a substantial improvement over qualitative detection methods used in the previous studies of breast MRS.

For quantitation of MRS data, AMARES [10], widely used quantitation tool was employed to analyze malignant breast spectra in this work. This advanced quantitation toolbox analyzes spectra in the time domain utilizing parametric prior knowledge (i.e., amplitude, phase linewidth, frequency, and shape). The present study used a narrow frequency range (i.e., 0.6 ppm) to fit small Cho peak without bias from neighboring lipid resonances. Soft constraints were imposed for a faster and more accurate quantitation during spectral fitting.

The aim of this study was twofold, firstly to demonstrate the feasibility of internal reference method for quantifying the Cho concentrations in patients with malignant breast tumors using a clinical 1.5 T scanner, and secondly to compare the range measured with the current method with previously published results.

Methods

Instrumentation

Phantom and in vivo studies were performed on a clinical 1.5 T whole-body system (Eclipse; Philips Medical System, Inc, Netherlands) with the standard ^{1}H-MRS acquisition software provided by the manufacturer. A body coil was used for transmission, and a dedicated four channel phased-array breast coil (USA Instruments, Aurora, OH, USA) was used for both MR imaging and MR spectroscopy. Home-built phantoms were prepared for in vitro quantification of the metabolite in the phantoms by ^{1}H-MRS using the fully relaxed water peak as an internal reference. Four choline chloride (Sigma-Aldrich, St. Louis, MO, USA) phantoms with known concentrations of choline chloride in pure water were used; the concentrations of choline chloride were 0.72, 1.44, 2.88, and 5.76 mmol/kg. Choline chloride was selected as the reference compound because this metabolite is the most important tumor marker.

In vitro study

After the localizer scan, coronal image using T_1-weighted spin echo sequence (TR/TE = 1,100/12 ms) was used for placing the volume of interest (VOI) in the phantoms before MRS. The voxel size was $2 \times 2 \times 2\,\mathrm{cm}^3$ in the phantom. For localization, spectra were obtained with a point-resolved spin-echo sequence (PRESS) [11, 12]. Shimming was performed automatically on the water resonance for optimization of the homogeneities in each VOI, and typically water peak linewidths of 1–2 Hz (full width at half-maximum; FWHM) were achieved on the phantoms. After shimming procedure spectra were acquired with water suppression by applying three preceding chemical-shift-selective saturation (CHESS) pulses [13, 14], and the bandwidth of each CHESS pulse was 60 Hz. The following acquisition parameters were used: TR = 2,000 ms, TE = 270 ms, 128 acquisitions, spectral width = 1,953 Hz, and 2,048 data points. A fully relaxed, unsuppressed spectrum was also acquired to measure the amplitude of the water peak in the localized volume. No line broadening was applied before Fourier transformation. Manual zero and first-order phase corrections without baseline correction were applied and peak integration was performed on processed spectra using jMRUI [15] version 2.1 package (EU Project 'Advanced Signal Processing for Medical Magnetic Resonance Imaging and Spectroscopy', TMR, FMRX-CT97–0160).

The absolute quantification of choline concentrations in the breast phantoms using the water peak as an internal reference was studied before the in vivo measurements. This internal reference method for quantifying Cho in the breast in vivo was originally presented by Bolan et al. [7] at 4.0 T. The current work is an implementation of the same technique at clinical field strength of 1.5 T. In the present study, all acquisitions were recorded at maximum receiver gain which made corrections for different receiver setting unnecessary. Hence, the absolute choline concentration was calculated by the following equation (1):

$$[\mathrm{Cho}] = \frac{n_{\mathrm{H_2O}}}{n_{\mathrm{Cho}}\,\mathrm{MW}_{\mathrm{H_2O}}} \times \frac{S_{\mathrm{Cho}}}{S_{\mathrm{H_2O}}} \times \frac{f_{T_{1\mathrm{H_2O}}}}{f_{T_{1\mathrm{Cho}}}} \times \frac{f_{T_{2\mathrm{H_2O}}}}{f_{T_{2\mathrm{Cho}}}}, \tag{1}$$

where [Cho] is the concentration of the choline in the breast phantom; S_{Cho} is the signal amplitude of choline in the phantom; $S_{\mathrm{H_2O}}$ is the signal amplitude of the unsuppressed water in the localized spectrum. n_{Cho} and $n_{\mathrm{H_2O}}$ are the numbers of ^{1}H nuclei for each molecule, respectively. The ratio of S_{Cho} and $S_{\mathrm{H_2O}}$ amplitudes can be changed to molal concentration by correcting for the number of ^{1}H nuclei per molecule and, molecular weight of the solvent, $\mathrm{MW}_{\mathrm{H_2O}}$. The f_{T_1} and f_{T_2} relaxation factors were corrected by using the equation for relaxation times:

$$f_{T_1} = 1 - \exp\left(-\frac{\mathrm{TR}}{T_1}\right), \tag{2}$$

$$f_{T_2} = \exp\left(-\frac{\mathrm{TE}}{T_2}\right). \tag{3}$$

For the estimation of T_1 and T_2 relaxation times, we measured the values of T_1 for choline and water in the breast phantom by performing a localized PRESS experiment at a fixed TE value (270 ms) and using five values of TR (1.5–8.0 s). We also measured the value of T_2 using five TE values (68 – 600 ms) at a fixed TR value (2 s). The values of T_1 and T_2 were obtained by

fitting the data to a mono-exponential model as a function of TR and TE, respectively. After the T_1 and T_2 corrections were made, the level of choline metabolite in this work was calculated as a concentration in units of mmol/kg.

In vivo study

Forty-five patients (range 31–73 years old, mean 50 years) with biopsy-confirmed breast cancer who were scheduled to receive surgery or chemotherapy within 2 weeks were recruited to participate in this study between June 2004 and August 2005. The biopsied lesions were considered malignant with a histological diagnosis of ductal, lobular, and in-situ cancer. This study was approved by the Institutional Review Board at the University of Irvine School of Medicine. Informed written consent was obtained from each patient prior to the studies. Patients were referred to the study by medical oncologists, surgeons, or radiologists at the University of Irvine or at private practice. All patients were examined with the same MRI/MRS protocol after biopsy, which consisted of high-resolution imaging, and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and single-voxel ^{1}H-MR spectroscopy. The typical delay between biopsy and this study was approximately 2 weeks.

Data acquisition: DCE-MRI and single-voxel ^{1}H-MRS

All patients were examined in prone position, and the breasts were gently cushioned with rubber foam to reduce patient motion. After the localizer scan, sagittal view T_1-weighted pre-contrast images were acquired from the breast of concern, using a spin echo (SE) sequence with TR/TE 1,000/12 ms, matrix size 256×256, field of view (FOV) 22 cm, and 34 slices with 3 or 3.5 mm thickness. Following this, a 3D SPGR (RF-FAST) pulse sequence with 16 frames (repetitions) was prescribed for dynamic imaging. Thirty-two axial slices with 4 mm thickness and no gap were used to cover bilateral breasts. The imaging parameters were TR/TE 10 ms/3.6 ms, flip angle 20°, acquisition matrix size 256×128, and FOV varying between 32 and 38 cm. The scan time was 42 s per acquisition. The sequence was repeated 16 times for dynamic acquisitions, 4 pre-contrast and 12 post-contrast sets. The contrast agent (Ominscan®, Amersham Health AS, Oslo, Norway; 0.1 mmol/kg body weight) was manually injected at the beginning of the 5th acquisition, and was timed to finish in 12 s to make the bolus length consistent for all patients. Immediately following the contrast, 10 cc saline was used to flush the contrast medium. After all 16 frames were acquired, the contrast-enhanced subtraction images at different times after injection can be obtained by subtracting the mean pre-contrast images (averaged over frames 1–4) from post-contrast enhanced images at a different time. In this study enhancement images obtained from the 6th frame (i.e., at 1-min after contrast injection) were used to ensure a correct localization of the VOI in the tumor for the MRS study.

Localized single-voxel ^{1}H MR spectra were acquired from the enhanced breast lesion for each patient in this study. The spectroscopic voxel was carefully positioned to maximize the coverage of the contrast-enhanced lesions and minimize the inclusion of adipose tissue. PRESS sequence [11, 12] was used for a correct localization of the volume on the centered lesion of interest. Voxel shimming was performed automatically on the water resonance for optimization of the homogeneities of the localized volumes of the breast, and FWHM was usually 8–17 Hz. The suppression of water was performed by using three CHESS pulses [13, 14], and fat signal was attenuated by using frequency-selective lipid suppression. The PRESS spectral parameters were used with a TE of 270 ms, TR of 2,000 ms, and acquisition averages of 128. A fully relaxed, unsuppressed spectrum (24 averages) was also acquired to measure the water and lipid peaks.

Analysis: preprocessing and fitting

The jMRUI software package [15] provides a graphical user interface to perform time-domain analysis of in vivo MR data. For the unsuppressed spectra used to measure the water and lipid peaks, each free induction decay signal was truncated to 1,024 points and then zero-filled to 2,048 points. After Fourier transformation, automatic (or manual) phasing was used to correct every signal with the zero-order phase of its water peak. Maximum peak of the water signal was assigned to 4.7 ppm, and the polymethylene lipid peak was at 1.32 ppm. We selected frequency ranges (i.e., water = 4.2–5.2 ppm, lipids = 0 – 1.8 ppm) of the spectrum for preprocessing and quantitation. In order to measure the Cho peak from the suppressed spectrum, we performed a preprocessing that consisted of truncation of 1,024 points, zero-filling of 4,096 points, Gaussian apodization of 5 Hz, Fourier transformation, and phase correction of the transformed spectrum. The narrow frequency range for analyzing Cho peak (i.e., 2.92 – 3.52 ppm) was selected to quantify its amplitude.

AMARES (Advanced Method for Accurate, Robust and Efficient Spectral fitting) [10], a widely used quantitation tool for MRS data was employed to analyze the spectra obtained from breast lesions at 1.5 T. AMARES, which is part of the jMRUI package, processes data in the time domain, and offers only Lorentzian or Gaussian lineshapes for a given peak as the prior knowledge. In this work, a Gaussian lineshape model was chosen for quantifying the Cho peak. The peak was fitted by minimizing the residuals over a 0.6 ppm section of the spectrum centered on the peak. After the zero and first-order phases were switched off, the frequency-selective option [16] was applied, weighting the first 20 points of the time domain signal by the first quarter of a squared sine function. Application of the frequency-selective option was used to reduce the effects of broad baseline components. The fitting error was estimated using the Cramer-Rao lower bound (CRMVB) of the signal amplitude (S) [17]. The CRMVB is not actually an estimate of the fitting error but it is rather a theoretical minimum limit for the estimation accuracy. Therefore, the error was expressed as a normalized standard deviation (SD): $\sigma_S = \sqrt{\mathrm{CRMVB}_S}/S$. In the water unsuppressed spectra, water peak was fit at 4.7 ppm, and the polymethylene lipid peak was fit at 1.32 ppm, respectively.

In vivo measurement of Cho in malignant breast tumors

T_1 and T_2 values for choline-containing compound in four patients with the malignant breast lesions were examined by a localized PRESS sequence experiment, respectively. T_1 values were estimated using a fixed TE (270 ms) and three TR values ranging from 1.5 to 8.0 s. T_2 values were estimated using a fixed TR (2 s) and three different TEs (150, 350, and 450 ms). T_1 and T_2 values were obtained by fitting the data to a mono-exponential model as a function of TR and TE to a mono-exponential model, respectively. In this work the averaged T_1 and T_2 values (mean ± SD) for Cho and water were used for correction. The concentration of in vivo Cho in malignant breast tumors was calculated with Eq. (1), and was expressed as a concentration in units of mmol/kg. This measurement error was expressed as a normalized error multiplied by the calculated concentration, $\sigma_S \cdot [\text{Cho}]$.

Results

In vitro study

The concentrations of choline in the breast phantoms were determined according to Eq. (1), and the calculated concentrations (mmol/kg) were displayed with the known concentrations of choline chloride (mmol/kg) in pure water of the four phantoms, as shown in Fig. 1. The values of T_1 for choline and water were determined to be within a range of 2,301–2,415 ms and 2,574–2,619 ms, respectively; and the values of T_2 for choline and water were determined to be within a range of 485–664 ms and 1,122–1,282 ms, respectively. There was a high correlation between the absolute measurements by ^{1}H-MRS and the known concentrations ($r^2 > 0.98$). In vitro measurement indicates that it is possible to use the fully relaxed water signal as an internal reference for the calculations of the metabolite concentrations. Therefore, we infer from the in vitro results that the method is applicable for the in vivo measurement as well.

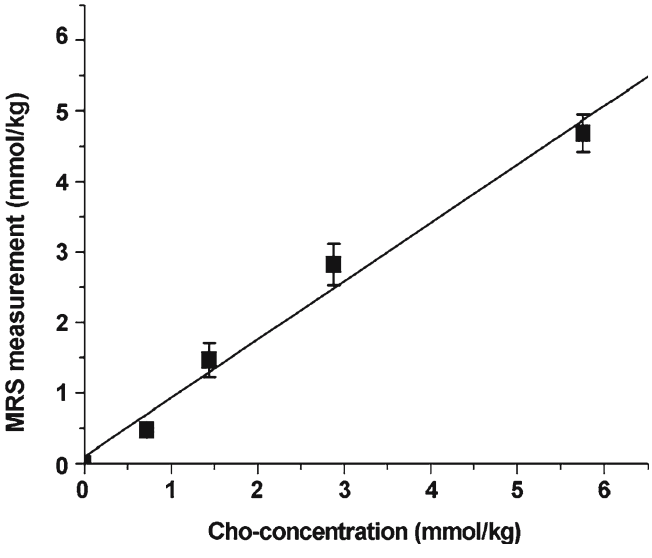

Fig. 1 The linearity between the absolute choline concentrations by MRS measurement using water as an internal reference and the known concentrations of choline in pure water of the four phantoms ($r^2 > 0.98$)

In vivo study

Forty-seven MR spectra were acquired from 45 patients with biopsy-confirmed breast cancer. In all patients, cancerous lesions showed contrast enhancement in the DCE-MR imaging. The mean size of these tumors was 3.40 cm (range 1.0–8.5 cm) measured as the largest dimension of the breast lesions in the axial subtraction images. The spectroscopic voxel size was either 5.8 or 8.0 ml depending on the size of the tumor. A mean Cho resonance was detected at 3.22 (range 3.17–3.26) ppm in 34 (72%) of the 47 spectra. The Cho peak was fitted with a Gaussian, and each unsuppressed water and lipid peak was fitted with a Lorentzian. The fitted Cho peak had a Gaussian linewidth of 1.5–7 Hz, and the fitted water peak had a Lorentzian linewidth of 7–24 Hz; the 1.32 ppm lipid peak had 11–46 Hz at 1.5 T.

To calculate the absolute Cho concentration, we measured the relaxation values in four patients with malignant breast tumors. Figure 2 illustrates the intensity variation, with respect to TE and TR, of the Cho peak from a malignant breast lesion in one patient. Figure 2a presents spectra obtained at TEs of 150, 350, and 450 ms using a fixed TR of 2 s. The averaged T_2 relaxation times (mean ± SD) were $\text{Cho} = 269 \pm 61$ ms and $\text{water} = 97 \pm 10$ ms from the four breast patients by fitting the data to a mono-exponential model (the value of $r^2 > 0.99$), respectively. Figure 2b displays spectra obtained using values of TR of 1.5, 4, and 8 s, respectively, at the same value of TE (270 ms). The averaged T_1 relaxation times for Cho and water were 1, 513 ± 156 ms and 746 ± 118 ms, respectively. The T_1 value of Cho in this work is reasonably consistent with a brain tissue literature value for choline of 1,500 ms [18].

After the T_1 and T_2 corrections were made, the absolute Cho levels had a range of 0.76–21.20 mmol/kg in 34 malignant breast spectra. Figure 3 shows a plot of Cho concentration versus lesion size. The Cho levels did not appear to be related to lesion sizes of cancer (i.e., invasive ductal and lobular carcinoma). Figures 4 and 5 demonstrate representative MRI and MRS on patients diagnosed by histology with breast cancer. Figure 4 shows a 63-year-old patient with an invasive ductal carcinoma. The spectroscopic voxel ($2 \times 2 \times 2\,\text{cm}^3$) was carefully positioned to maximize the coverage of the hypointense lesion on the

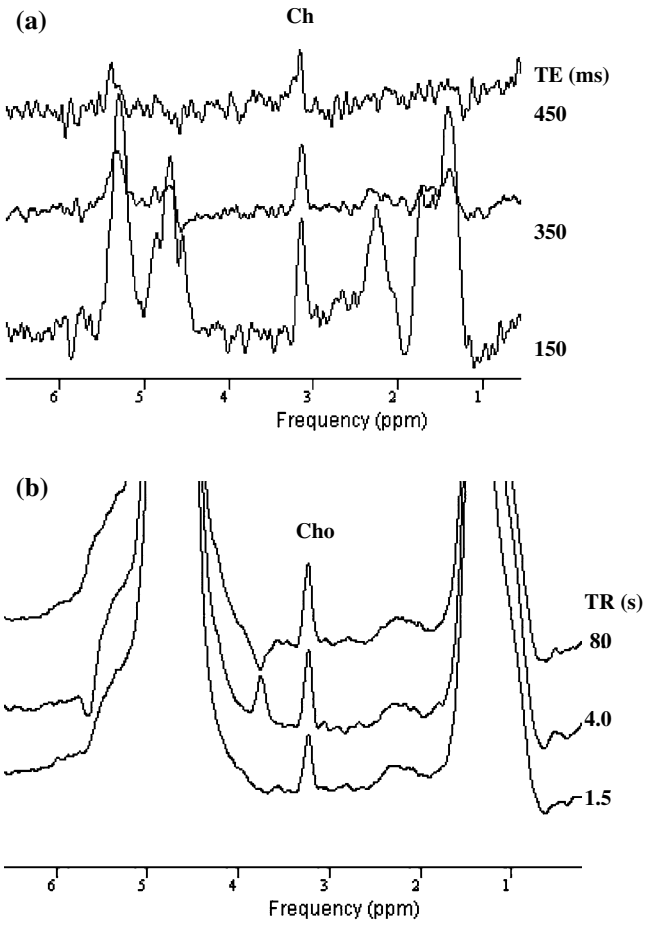

Fig. 2 T_2 and T_1 values for choline-containing compounds (Cho) in malignant breast tumor were measured by a localized PRESS experiment with volume of interest (size, $2 \times 2 \times 2\,cm^3$) placed centrally in the enhanced breast lesion. Spectra obtained from values of TE of 150, 350, and 450 ms at the same value of TR (2,000 ms) (**a**). Spectra obtained from values of TR of 1,500, 3,500, and 8,000 ms at the same value of TE (270 ms) (**b**)

centered sagittal image (Fig. 4a) and on the contrast-enhanced lesion in the subtraction axial image (Fig. 4b). Elevated Cho peak at 3.23 ppm is clearly visible in the water-fat suppressed spectrum. The Gaussian model fitting of the Cho peak produces a measurement of [Cho] = 19.24 ± 0.85 mmol/kg and the estimated model fit is shown above the full spectrum and the residue is shown underneath (Fig. 4c). Figure 5 shows a 69-year-old patient with an invasive ductal carcinoma. On the pre-contrast sagittal image one lesion showed hypointense signal with round shape, approximately 1 cm in the lower left breast. The spectroscopic voxel (size, $1.8 \times 1.8 \times 1.8\,cm^3$) was positioned to minimize the inclusion of adipose tissue (Fig. 5a) and include the contrast-enhanced lesion in the subtraction axial image (Fig. 5b). Figure 5c is a spectrum obtained from the small lesion in a selected voxel, with [Cho] = 10.44 ± 7.43 mmol/kg.

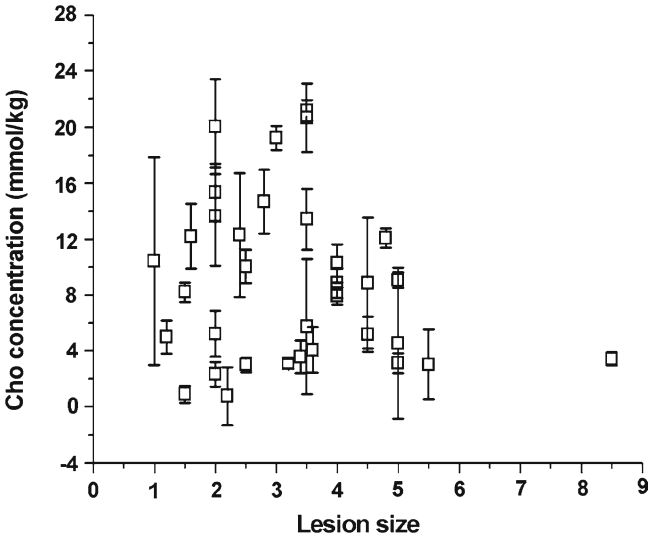

Fig. 3 This figure shows a plot of Cho concentration versus lesion size obtained from 34 malignant breast lesions. The absolute Cho levels had a range of 0.76–21.20 mmol/kg (mean = 8.90 mmol/kg), and did not appear to be related to lesion sizes (mean = 3.40 cm) of cancer (i.e., invasive ductal and lobular carcinoma)

Discussion

In vivo ^{1}H-MRS is a non-invasive technique that provides information on tumor metabolism, showing a substantial elevation of choline-containing compounds [19, 20]. Several studies have demonstrated that Cho can be detected in human breast tumors using ^{1}H-MRS at 1.5 T [1–6]. However, all of these studies used qualitative methods [i.e., detectibility or Cho signal to noise ratio (SNR)] for the differentiation of malignant and benign lesions. It is obvious that a more general method is necessary for improving the accuracy of lesion diagnosis because the sensitivity of the MRS measurement is variable due to variations in voxel size, adipose tissue content, and receiver coil efficiency. The present study applied an internal reference method to investigate the absolute quantification of Cho levels in malignant breast tumors. We demonstrated that quantitative ^{1}H-MRS conducted using a clinical 1.5 T scanner can be used for quantifying Cho levels accurately in breast lesions.

In other previous breast ^{1}H-MRS studies, several groups have also proposed in vivo absolute quantitation using an external reference method with known concentrations [1, 9]. They demonstrated that this method can be used for determining Cho concentrations in breast lesions. However, these studies did not correct for partial volume of adipose tissue in the voxel. On the other hand, another group more recently used an internal reference method for quantifying Cho levels in breast tissue at 4.0 T [7]. These authors suggested in that work that an internal ref-

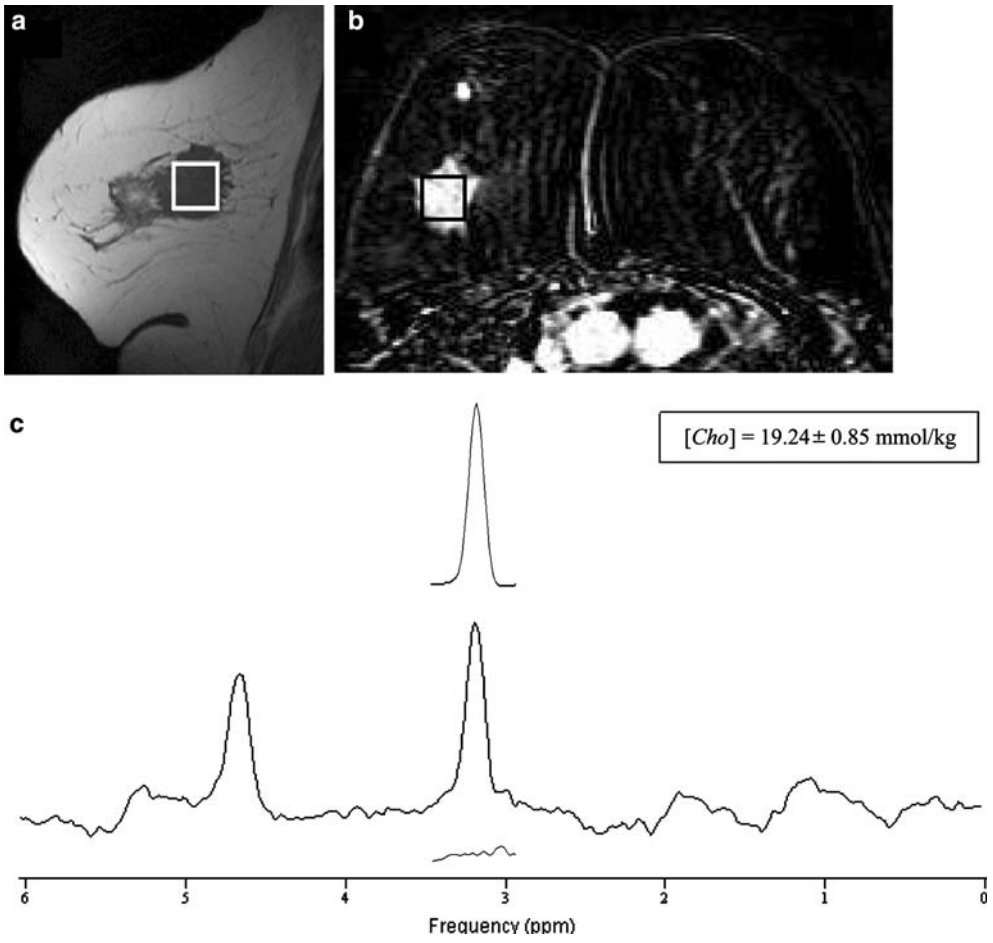

Fig. 4 MRI and MRS measurement in a 63-year-old patient with invasive ductal carcinoma. On pre-contrast sagittal image one lesion showed very dark intensity signal with irregular shape in the outer right breast. The lesion size in the superior-inferior direction was approximately 3 cm. The spectroscopic voxel (size, $2 \times 2 \times 2\,cm^3$) is superimposed on the hypointense lesion in the pre-contrast sagittal image (**a**) and on the contrast-enhanced lesion in the subtraction axial image (**b**). The MR spectrum was measured from the selected lesion. A Cho peak at 3.23 ppm is clearly visible in the water-fat suppressed spectrum (**c**). The Gaussian model fitting of the Cho peak produces a measurement of $[Cho] = 19.24 \pm 0.85$ mmol/kg, and the estimated model fit is shown above the full spectrum and the residue is shown underneath

erence method can significantly improve the previously reported analysis methods by accounting for the variable sensitivity of breast ^{1}H-MRS measurements as this internal method using water as a reference automatically compensates for the partial volume effect, and does not require separate calibration experiments. Moreover, the variation in water content may be quite large, depending on the placement of the voxel, but the internal referencing corrects for this. The internal method assumes that water content does not change during varying pathological conditions. For this reason, an internal reference method was chosen in the present study to see if the method could provide accurate results at 1.5 T. We demonstrated the potential of absolute quantification using an internal reference for the measurement of Cho concentrations from in vitro experiments and in vivo breast lesions. Furthermore, the measurement of the internal reference method should be accurate and reproducible to avoid system errors in the calculation of Cho concentrations.

The present study used the AMARES quantitation tool [10] for accurate spectral fitting of MR malignant breast spectra. The AMARES method is based on the time domain fitting technique utilizing parametric prior knowledge. In this work, we used a narrow frequency range (0.6 ppm) to fit small Cho peaks without bias from neighboring lipid resonances. Soft constraints were also imposed for a faster and more accurate quantitation during spectral fitting. Linewidths for Cho peak were allowed to vary between 1 and 10 Hz. The frequency constraint range was restricted to ±0.2 ppm (i.e., 3.12–3.32 ppm). In our analysis a Gaussian lineshape model was chosen exclusively for quantifying the Cho peak because patient's motion and respiration caused peak broadening that led to Gaussian lineshapes in the averaged spectra. This model performed reasonably well in our malignant breast spectra, and showed small standard deviations in fitting errors (Fig. 4c). Hence, the use of Gaussian lineshapes may be useful for fitting a single Cho peak acquired from malignant breast lesion. In addition, the lineshapes of typical human brain spectra at 1.5 T also exhibited only a small deviation from Gaussian lineshape [21].

Early studies have reported quantitative Cho levels in malignant breast lesions using ^{1}H-MRS at 1.5 T. Roebuck et al. [1] found that detectable levels of Cho were 0.4–5.8 mmol/l in seven patients with malignant lesions and their detection threshold was 0.2 mmol/l. Bakken

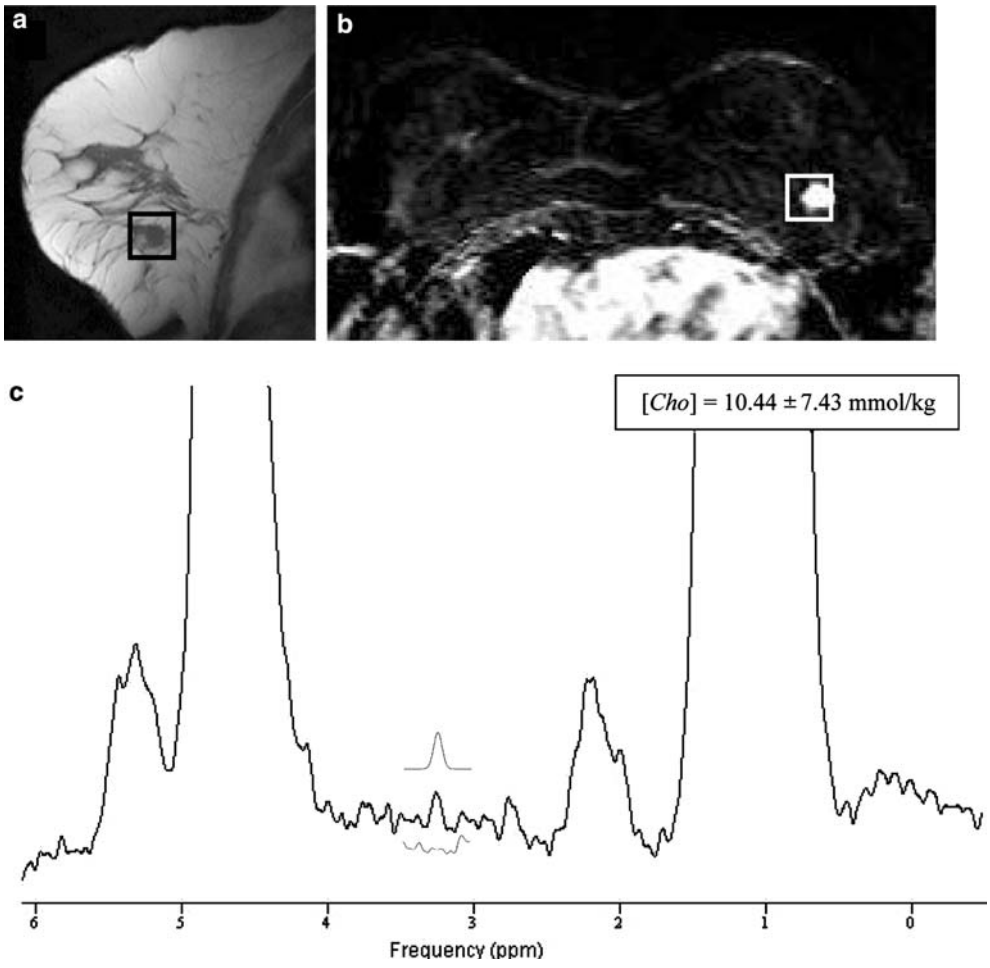

Fig. 5 MRI and MRS measurement of a 69-year-old patient with invasive ductal carcinoma. On pre-contrast sagittal image one lesion showed hypointense signal with round shape, approximately 1 cm in the lower left breast. The spectroscopic voxel (size, $1.8 \times 1.8 \times 1.8\,cm^3$) encompassed the hypointense lesion in the pre-contrast sagittal image (**a**) and the contrast-enhanced lesion in the subtraction axial image (**b**). A Cho peak is visible at 3.22 ppm, and the fitting produces a measurement of [Cho] = 10.44 ± 7.43 mmol/kg (**c**)

et al. [9] found a single measurement of 2 mmol/l in a patient with malignant breast lesion, and the Cho level was in reasonable agreement with the value of their in vitro ^{1}H-MRS findings (1.2 mmol/l). Recently, Bolan et al. [7] found that the Cho levels had a range of Cho measurements of 0.4–10 mmol/kg in a number of breast tissue spectra using 4.0 T. Their threshold for malignancy was 1.38 mmol/kg. In our study, the measured Cho levels were within a range of 0.76–21.20 mmol/kg from 32 patients with malignant breast tumors, which is rather consistent with the findings of previous studies above. Further investigations are needed to determine whether the absolute Cho values reported here are reasonable and reproducible in repeated MRS measurements. In addition, several correction factors must be taken into account, among them calibration constants depending on the particular referencing method used, corrections for in vivo and in vitro T_1 and T_2 relaxation effects, hardware factors, voxel size, and pulse sequence dependent parameters, etc. For ^{1}H-MRS using 1.5 T, further studies are also needed to determine a threshold Cho value for differentiating between malignant and benign lesions.

While most researchers believe that there is a direct relationship between total choline concentration and degree of malignancy, the recent work by Stanwell et al. [22] has shown that the location of the choline peak is also related to malignancy. This work performed at 1.5 T has shown that those subjects who had their choline peak at 3.22 ppm had cancer whereas those who had their peaks at 3.28 ppm were either false-positive volunteers or lactating mothers. It should be emphasized that the determination of the exact location of the choline peak requires precise referencing. Referencing based on the location of a methylene peak at 1.33 ppm, as done by Stanwell et al., could be problematic due to the very broad lipid peak in the human breast. In spite of this, the results by Stanwell et al. prove to be interesting and deserve additional investigation to confirm this finding.

In vivo ^{1}H-MRS studies have shown that elevated Cho peak at 3.2 ppm is observed in neoplastic tissues [1–6]. However, the precise mechanism for elevated Cho is not completely understood, and still remains unclear. Recently, high resolution ^{1}H-NMR spectra acquired from biopsy tissues have shown that a Cho resonance peak

actually is comprised of multiple signals, such as phosphocholine, glycerophosphocholine, and free choline [23–26]. Among these signals, the primary component contributing to the Cho peak is phosphocholine, a known precursor of cell membranes synthesis [27–31]. Thus, in this work, the elevated Cho level in neoplastic tissues may be a reflection of the increased membrane synthesis by replicating cells. For further interpretation, it is necessary to understand a typical alteration between biosynthetic pathways and catabolic breakdown pathways in which Cho serve as both precursors and catabolites [32].

In conclusion, the present study demonstrated the feasibility of absolute quantification for measuring Cho concentrations in malignant breast tumors by ^{1}H-MRS using water as an internal reference at 1.5 T. The calculated Cho levels in this work were rather consistent with the previous in vivo estimates. Therefore, it is expected that quantitative MRS measurement using clinical 1.5 T scanners might be of value for improving the accuracy of MR imaging in diagnosing breast tumors.

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