In Vivo Quantification of Choline Compounds in the Breast With 1H MR Spectroscopy

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This work describes a methodology for quantifying levels of total choline-containing compounds (tCho) in the breast using in vivo 1H MR spectroscopy (MRS) at high field (4 Tesla). Water is used as an internal reference compound to account for the partial volume of adipose tissue. Peak amplitudes are estimated by fitting one peak at a time over a narrow frequency band to allow measurement of small metabolite resonances in spectra with large lipid peaks. This quantitative method significantly improves previously reported analysis methods by accounting for the variable sensitivity of breast 1H MRS measurements. Using this technique, we detected and quantified a tCho peak in 214 of 500 in vivo spectra. tCho levels were found to be significantly higher in malignancies than in benign abnormalities and normal breast tissues, which suggests that this technique could be used to diagnose suspicious lesions and monitor response to cancer treatments. Magn Reson Med 50:000–000, 2003. © 2003 Wiley-Liss, Inc.

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Breast cancer is a very common disease, affecting 11% of American women and causing more than 40 000 deaths each year (1). While breast cancer mortality is decreasing, the incidence continues to rise (2). Thus, there is a great need for noninvasive diagnostic tools for both screening and treatment monitoring. The conventional diagnostics—X-ray mammography, sonography, and physical examination—are limited in their sensitivity for detecting disease and their specificity for distinguishing between benign and malignant lesions. Magnetic resonance imaging (MRI) of the breast is being used increasingly because of its high sensitivity, but its reported specificity is widely variable (3).

Researchers have recently begun to augment breast MRI studies with MR spectroscopy (MRS) to increase specificity. In vivo MRS can detect a resonance at 3.25 ppm that has contributions from several different compounds, including choline, phosphocholine, glycerophosphocholine, and taurine. High-resolution in vitro and ex vivo studies indicate that the levels of choline compounds increase with malignancy (4–6). At the lower field strengths used for in vivo work (1.5–4 T), these multiple resonances cannot be spectrally resolved and thus appear as a single peak, termed total choline-containing compounds (tCho).

Several studies conducted at 1.5 T have shown that in vivo MRS can be used to distinguish between benign and malignant tissues (7–11). These studies used the hypothesis that tCho is only detectable in malignancies. A pooled analysis of these five studies showed that this tCho detectability criterion can identify malignancies with an 83% sensitivity and 85% specificity (12). This qualitative approach is promising, but it is only applicable if the MRS measurement sensitivity is invariant. In similar studies performed at 4 T, the increased sensitivity allows detection of tCho in benign lesions and normal subjects. A more general approach is to quantify the tCho peak with the expectation that tCho levels are higher in malignancies than in benign lesions or normal tissues. Two groups have reported quantification of tCho levels using external phantom referencing methods (7,13). These studies demonstrated the feasibility of quantitative breast MRS, but they were limited to small patient groups and did not report measurement errors. Quantification is also valuable for measuring tumor response to treatment regimens. Two other studies found that the detectability and amplitude (judged qualitatively) of the tCho peak decreases after chemotherapy (8,11). A quantitative method enables finer measurements of the magnitude and rate of tumor response.

Although quantification of metabolite levels is routinely performed in MRS of the brain, it is more difficult to perform in the breast because of the heterogeneous distribution of the glandular and adipose tissues. Although the spectroscopist will typically plan a voxel to include mainly glandular tissue and tumor, voxels of typical size (1–2 mL) nearly always contain some adipose tissue as well. The amount of included adipose tissue can vary greatly depending on the architecture of the gland and/or lesion.

The two basic elements of a quantitative MRS methodology are the referencing strategy and the spectral fitting technique. The referencing strategy proposed in this work uses water as an internal reference peak. This approach compensates for the partial volume of adipose tissue in the voxel and naturally leads to a molal (mol/kg) concentration for water-soluble metabolites. The fitting technique used in this work is based on the time domain/frequency domain (TDFD) approach (14). This method enables flex-
ible lineshape definition by the use of a TD model, and has excellent frequency-selection properties since the residuals are evaluated and minimized in the FD. The ability to select a narrow frequency range is crucial for fitting small resonances in the presence of very large ones, as is the case in breast spectra containing large lipid peaks.

The goal of this project was to develop a method to quantitatively measure tCho levels in breast tissue. The methodology presented integrates several existing techniques: single-voxel localization using LASER (15), TE averaging to reduce lipid sideband artifacts (16), automatic frequency referencing to correct respiration artifacts, frequency-selective spectral fitting, and quantification using water as an internal reference peak. We quantified 500 in vivo spectra with this technique, analyzed the results, and compared them with an independent method based on external referencing. The applicability of using quantitative \(^1\)H MRS for diagnosing suspect breast lesions is discussed below.

MATERIALS AND METHODS

Acquisition

All measurements were performed with a hybrid 4 T system, consisting of a 90-cm-bore magnet (model 4 T-900; Oxford Magnet Technology, Oxfordshire, UK) with a clinical gradient system (Sonata; Siemens, Erlangen, Germany) interfaced with an imaging spectrometer (Unity Inova; Varian, Palo Alto, CA). Several different single-breast quadrature transmit/receive RF surface coils of similar design were used to accommodate different breast sizes. The coils were mounted on a custom-built patient table designed for unilateral, prone breast studies.

A total of 105 subjects (23–72 years old, mean = 48 years) were studied in 175 MRI/MRS sessions. Of these, 86 were participants in a study involving the diagnosis of lesions with suspicious mammographic findings, 14 were participants in a study regarding monitoring response to neoadjuvant chemotherapy, and five were presumed normal volunteers (no breast-related health problems or abnormal mammograms). Approximately half of these studies were performed after a needle biopsy or other invasive procedure. All studies were approved by the institutional review board, and informed written consent was obtained from the subjects prior to the studies.

All 100 patients from the diagnosis and treatment-monitoring studies were examined with the same MRI/MRS protocol, which consisted of high-resolution imaging, dynamic contrast-enhanced imaging, and single-voxel spectroscopy. The subjects were positioned prone with their breast centered horizontally in the magnet. After scout images were acquired to verify position, the coil was manually tuned and matched. A high-resolution 3D fast low-angle shot (FLASH) image (fat-suppressed, matrix = 256 \times 256 \times 64, field of view (FOV) = 14–18 cm, TE/TR = 4.1/13.5 ms, flip angle = 30°) and a fast 2D multislice image (fat-suppressed, matrix = 256 \times 128, 30 slices, slice thickness = 2.5 mm, FOV = 14–18 cm, TE/TR = 5.1/390 ms, flip angle = 90°) were acquired prior to injection of Gd-DTPA (0.1 mmol/kg body weight). Five to seven 2D images were acquired immediately after injection, followed by a second 3D image. Both image sets were analyzed with our own image-processing software (developed with Matlab [The Mathworks, Natick, MA]) to select voxels for MRS with the subject still in the magnet. The criteria for voxel selection included lesion architecture, dynamic Gd-DTPA uptake, and prior clinical information from mammographic or ultrasound images. The voxels were planned to maximize coverage of the lesion and minimize the inclusion of adipose tissue. The five normal subjects were studied with only the high-resolution 3D FLASH image followed by spectroscopy, since no Gd-DTPA was administered in these studies.

All spectra were acquired using the LASER localization technique (15) with 4096 complex points and 6-kHz spectral width. Each voxel measurement began with a calibration of the localized B1 field strength, followed by 30–60 s of manual adjustment of the linear shims. A fully relaxed, single-shot, unsuppressed spectrum was acquired to measure the water and lipid peaks. The power required to suppress the water signal using VAPOR (17) suppression was then manually adjusted. The metabolite spectrum was acquired using TE averaging with TE = 45–196 ms in 64 or 128 increments and with TR = 3 s (16). Each free induction decay (FID) signal was individually saved, and no averaging was performed until processing. Each voxel required ~9 min in total. One to four voxels were studied in each subject, for a total acquisition time of ~1 hr.

Preprocessing Spectra

All spectral processing programs, including preprocessing, fitting, and quantification, were written in Matlab (The Mathworks, Natick, MA). In all spectra, the last 512 points of the raw TD FID were used to calculate the root-mean-square (RMS) noise and correct for DC offsets. For the unsuppressed spectra used to measure the water and lipid peaks, the FIDs were truncated to 1024 points and then zero-filled to 2048 points. The zero-order phase \( \phi \) was measured and corrected using the average of two different autophasing methods: 1) fitting the phase of the first few TD points to a line and using \( \phi \) at time \( t = 0 \), and 2) finding the value of \( \phi \) that maximizes the smallest value of the real part of the FD spectrum. Using the mean of these two methods produces a robust estimate of \( \phi \). The spectra were then frequency referenced by setting the maximum of the water peak to 4.7 ppm. For the water-suppressed spectra used to measure the metabolites, each individual spectrum from the TE averaging acquisition was automatically phased as described above. To correct respiration-induced frequency shifts, each spectrum was shifted in frequency to maximize the cross-correlation function between it and the first spectrum of the acquisition (18). After phasing and frequency correction was performed, the spectra were averaged.

Fitting

To fit the water, 1.3 ppm lipid, and tCho peaks in the processed spectra, a new fitting program was developed. The algorithm is based on the TDFD fitting technique proposed by Slotboom et al. (14). In this approach, a TD model is used to describe the peak parameters, but the fitting is performed by applying a Fourier transform and
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minimizing the residuals in the FD. This flexible fitting method has excellent frequency-selective properties and can be used with various lineshape definitions. In this implementation, a Voigt lineshape was used to model all peaks (19). The modeled signal from a single peak $s$ can be described in the TD $t$ as

$$s(t) = A \cdot \exp(-iat + ib - \lambda t - \gamma^2 t^2)$$

with signal amplitude $A$, chemical shift frequency $\omega$, zero-order phase $\phi$, Lorentzian damping factor $\lambda$, and Gaussian damping factor $\gamma$. The Lorentzian and Gaussian damping factors are related to the full width at half maximum (FWHM) of an impulse response: $\lambda = \pi \cdot \text{FWHM}$ and $\gamma = \pi \cdot \text{FWHM}/(2\sqrt{\ln 2})$, as described by Ogg et al. (20). Note that although all equations in this section are shown with continuous time and frequency for clarity, all calculations were performed with discrete variables.

The peaks were fit one at a time by minimizing the FD residuals over a 0.4-ppm (68-Hz) section of the spectrum centered on the peak. The zero-order phase $\phi$ was fixed at zero (since it was presumably corrected during preprocessing) and only the real portion of the residual was minimized. Initial values for parameters were generated based on prior knowledge and simple heuristics (e.g., the initial frequency is that of the maximum of the absolute value spectrum over the fitting region). The nonlinear minimization of the residuals was performed using the optimization toolkit provided with Matlab (function lsqnonlin, using the large-scale model option).

In the unsuppressed spectra, water was fit at 4.7 ppm, and the polymethylene lipid peak was fit at 1.3 ppm. In the suppressed and averaged spectra, tCho was fit at 3.25 ppm. The baseline around 3.25 ppm is often distorted, so a linear baseline model was used to reduce bias. Figure 1 shows the relationship between the data ($S_{\text{data}}$), model ($S_{\text{model}}$), baseline ($S_{\text{baseline}}$), and residual ($R$). Using a linear baseline, the residual function to be minimized is

$$R(\omega) = [S_{\text{data}}(\omega) - S_{\text{baseline}}(\omega) - \text{FFT}(S_{\text{model}}(t))]_{\omega = 0.2 \text{ppm}}$$

where $\omega_0$ is the center of the frequency range being fit. No baseline correction was used for fitting the water and lipid peaks, so in those cases $S_{\text{baseline}} = 0$ for all $\omega$.

The peak amplitude $A$ is the parameter of greatest interest since it is proportional to the number of nuclei in the voxel. The fitting error was estimated using the Cramer-Rao minimum variance bound (CRMBV) of the parameter $A$:

$$\text{CRMVB}_A = \frac{\sigma_{\text{noise}}^2}{\int_{-0.2 \text{ppm}}^{0.2 \text{ppm}} \frac{\partial}{\partial A} [\text{FFT}(s_{\text{model}}(t))] d\omega}$$

where $\sigma_{\text{noise}}^2$ is the variance of white, Gaussian noise. The CRMVB$_A$ is not actually an estimate of the fitting error; rather, it is a theoretical minimum limit for the estimation accuracy. Nevertheless, the CRMVB$_A$ is commonly used in NMR applications to estimate fitting errors (21). Effectively, this measure is the noise variance scaled by the sensitivity of the model to changes in the parameter $A$. The error is often expressed as a normalized standard deviation (SD): $\sigma_A = \sqrt{\text{CRMVB}_A}/A$.

This error estimate was also used to establish the detection criteria. In all cases, a fit was initially performed and the parameter estimates and errors were calculated. If the fitting error for $A$ was greater than a specified threshold, then the fit was rejected and the resonance was considered undetectable. For all of the spectra reported in this paper, the threshold for the normalized error was unity: if $\sigma_A > 1$, the peak was considered undetectable. Although this threshold is statistically arbitrary, it is convenient and roughly corresponds to a signal-to-noise ratio (SNR) of 2–3.

Quantification

The spectral fitting produces an amplitude $A$ for each peak, expressed in arbitrary units (au). To standardize this measurement for use in both internal and external referencing schemes, several corrections must be made to account for experimental conditions and the physical properties of each species. The corrected amplitude $A'$ is:

$$A' = \frac{A}{f_{\text{gain}}/f_{\text{cal}}T_1 T_2}$$

with correction factors

$$f_{\text{gain}} = \frac{\text{gain}}{\text{gain}_0}$$

$$f_{\text{cal}} = \frac{B_1}{B_{1,0}}$$

$$f_{T_1} = 1 - \exp(-T R / T_1)$$
\[ f_T = \frac{1}{N} \sum_{j=1}^{N} \exp(-TE/T_2) \]  

where \(\text{gain}\) is the receiver gain, \(B_j\) is the local amplitude of the excitation radiofrequency field, TR is the pulse repetition time, and TE is the echo time. The receiver correction factor \(f_{\text{gain}}\) is necessary if the receiver gain is different in the suppressed and unsuppressed acquisitions. The coil receive efficiency factor \(f_{\text{coil}}\) was calculated by assuming the transmit and receive efficiencies are equal. The reference values \(\text{gain}_0\) and \(B_{j,0}\) are arbitrary, but must be used consistently when comparing values from different acquisitions. The \(T_1\) correction \(f_{T_1}\) is approximate, and is valid provided \(TE \gg TR\). With a TE-averaged acquisition, the \(T_2\) correction \(f_{T_2}\) is a summation over \(N\) acquisitions, each with different \(TE\) (16). Because it is impractical to measure relaxation properties in each voxel, constant values were assumed for all relaxation constants based on measurements in several subjects.

After these corrections are made, the signal amplitudes are proportional to the number of nuclei in the volume. The ratio of the tCho and water amplitudes can be converted to molar concentration (moles solute per mass solvent) by correcting for the number of \(\text{H}\) nuclei per molecule \(\eta\) and the molecular weight of the solvent \(\text{MW}_{\text{water}}\):

\[ [\text{tCho}] = \frac{A}{f_{\text{gain}}f_{T_1}f_{T_2}f_{\text{coil}}} \times \frac{f_{\text{gain}}f_{T_1}f_{T_2}}{A_{\text{water}}} \times \frac{\eta_{\text{water}}}{\eta_{\text{tCho}}} \frac{\text{MW}_{\text{water}}}{V_{\text{water}}} \]

Note that the coil efficiency factor \(f_{\text{coil}}\) cancels because both water and tCho come from the same volume of interest. This quantity \([\text{tCho}]\), expressed in units mmol/kg, is the metric proposed as an in vivo measure of the tissue level of choline-containing compounds in the breast. This measurement is presented along with the SD of the fitting error: \([\text{tCho}] \pm \sigma_\text{A} \times [\text{tCho}]\).

In spectra where no tCho peak was detected based on the \(\sigma_\text{A} > 1\) criterion, an additional procedure was performed to determine the sensitivity of the measurement. A simulated, noiseless spectrum containing a single peak at 3.25 ppm with a Gaussian FWHM of 15 Hz and amplitude \(A_{\text{sim}}\) was added to the preprocessed in vivo spectrum. The combined in vivo + simulated spectrum was then fit using the procedure described above, and the fitting error \(\sigma_\text{A}\) was calculated. This process was repeated with successively smaller simulated peak amplitudes, reduced 10% each iteration, until the simulated peak was no longer detectable. The smallest value of \(A_{\text{sim}}\) that led to a detectable tCho peak was then used to calculate a minimum detectable level (MDL) of tCho, \([\text{tCho}]_{\text{MDL}}\) using Eq. [9]. For these in vivo spectra where tCho is not detectable, the overall \([\text{tCho}]\) measurement is expressed as \(0 \pm [\text{tCho}]_{\text{MDL}}\).

To validate this proposed internal referencing scheme, the tCho level was also calculated independently using an external referencing scheme. In general, the corrected signal amplitude \(A'\) of a resonance is proportional to the number of nuclei \(n\) in the sample: \(n = \kappa_{\text{sys}}A'\). The system constant \(\kappa_{\text{sys}}\) (with units mol/au) accounts for the system-specific hardware and software. The value of \(\kappa_{\text{sys}}\) was calculated for our system in separate calibration experiments with a phantom of known concentration and measurable relaxation properties. The externally-referenced concentration of tCho can then be expressed in molar units (mol/kg) as

\[ [\text{tCho}]_{\text{ext}} = \left( \frac{A}{f_{\text{gain}}f_{T_1}f_{T_2}} \right) \frac{\kappa_{\text{sys}}}{\eta_{\text{tCho}}} \frac{1}{\rho_{\text{water}}} \frac{\text{MW}_{\text{water}}}{V_{\text{water}}} \]

where \(\rho_{\text{water}}\) is the water density, and \(V\) is the voxel volume. To compare the internal and external methods, the tissue water density was assumed to be 1 kg/L. The volume \(V\) was assumed to be the entire voxel volume, ignoring the effect of partial volume from adipose tissue.

**RESULTS**

The quantification method was applied to 500 spectra acquired from 105 subjects in 175 MR study sessions. The voxel size ranged from 0.4 to 16 mL, with a median volume of 1.6 mL. A peak near 3.25 ppm was detected in 214 (43%) of the 500 spectra. The processing and fitting processes required ~1 min per spectrum on a typical workstation. After we ran the fully-automated procedure on all 500 spectra and manually reviewed the results, six spectra required reprocessing with manual intervention to adjust phase and/or frequency referencing. Each water, lipid, and tCho peak was fit with a Voigt lineshape, giving both Lorentzian and Gaussian contributions to the linewidth. The median Lorentzian and Gaussian linewidths for the fit water peak were 12 Hz and 13 Hz, respectively; the 1.3 ppm lipid peak had 16 Hz and 28 Hz, and the tCho peak had 0 Hz and 14 Hz.

To calculate absolute tCho levels, relaxation values were measured in several subjects using standard spectroscopic techniques. The measured values with SDs were:

- \(T_{1,\text{water}} = 870 \pm 325 \text{ ms}, T_{2,\text{water}} = 60 \pm 7 \text{ ms}, T_{1,\text{lipid}} = 480 \pm 100 \text{ ms}, T_{2,\text{lipid}} = 69 \pm 12 \text{ ms}, T_{1,\text{tCho}} = 399 \pm 133 \text{ ms}, T_{1,\text{tCho}}\) was not measured, but was assumed to be the same as \(T_{1,\text{water}}\).

Although some of these SDs are large, their overall effect on the \([\text{tCho}]\) measurement is relatively small. Using our acquisition parameters, the uncertainties in \(T_{1,\text{water}}, T_{2,\text{water}}, T_{1,\text{tCho}}, \text{ and } T_{2,\text{tCho}}\) led to \([\text{tCho}]\) SDs of 0%, 5%, 6%, and 13%, respectively, for a total of 16%.

Several examples of in vivo spectra are shown in Fig. 2. Figure 2a shows a large voxel acquired in healthy glandular tissue. A clear tCho peak is visible at 3.25 ppm, and the fitting produces a measurement of \([\text{tCho}] = 0.75 \pm 0.07 \text{ mmol/kg}\). The model fit of this peak is shown above the full spectrum, and the smooth residual is shown beneath. There is another metabolite peak visible at 3.4 ppm that was not fit. This sample shows that tCho can be detected in normal breast tissue. Figure 2b is a spectrum from an invasive ductal carcinoma with \([\text{tCho}] = 6.8 \pm 0.09 \text{ mmol/kg}\). The volume of this voxel is smaller than that of the voxel in Fig. 2a, but since it is located closer to the coil the sensitivity is comparable. The SNR of the tCho peak is quite high, but the residual shows some structure, indicating an imperfect fit. Figure 2c shows a more typically-sized voxel in a lesion later identified by needle biopsy as atypical hyperplasia, with \([\text{tCho}] = 1.5 \pm 0.8 \text{ mmol/kg}\). Atypical hyperplasia is generally considered...
to be benign, but it is a marker indicating an increased risk for developing future malignancies. The low SNR of the tCho peak is reflected in the error estimated by the fitting procedure.

These examples demonstrate how the sensitivity of breast MRS can vary greatly. Due to variability in coil loading, voxel size, and partial volume of adipose tissue, the sensitivity for detecting tCho varied by a factor of 100 in this study. Figure 3 shows the fitting error in all 500 spectra as a function of water SNR and voxel volume. For both natural and simulated spectra, the fitting error is the normalized error multiplied by the calculated concentration, [tCho] · σA or [tCho]MDL · σA. Clearly, the water SNR is a better indicator of fitting error, since it automatically corrects for coil efficiency and the partial volume of adipose tissue. These plots show reasonable properties for an unbiased fitting method. For example, fitting error decreases uniformly with increasing water SNR, and tCho is more likely to be detected in spectra with lower fitting errors.

Although the fitting error is greater in smaller voxels, the [tCho] measurement itself is independent of the voxel size, as shown in Fig. 4a. The filled diamonds represent [tCho] measurements in spectra where a peak was detectable, and the hollow diamonds represent the MDL of tCho in spectra where no peak was detectable. As expected, the smaller

FIG. 2. Example spectra. A water-suppressed spectrum is shown on the right, with the tCho fit shown above and the residual (including linear baseline) underneath. The location of the voxel is shown in a contrast-enhanced, fat-suppressed sagittal image on the left. a: Normal gland, volume = 13.0 mL, [tCho] = 0.66 ± 0.06 mmol/kg, lipid fraction = 3%. b: Malignant tumor of invasive ductal carcinoma, volume = 6.8 mL, [tCho] = 6.1 ± 0.08 mmol/kg, lipid fraction = 8%. c: Benign finding of atypical hyperplasia in an insensitive region of the coil, volume = 1.1 mL, [tCho] = 1.4 ± 0.7 mmol/kg, lipid fraction = 14%.
voxels were less likely to have detectable tCho, but clearly the voxel size did not bias the \([t\text{Cho}]\) measurement in spectra with detectable tCho.

Figure 4b shows how the \([t\text{Cho}]\) measurement varies with the lipid content of the voxel. The lipid fraction is estimated using the ratio between the corrected amplitudes of the water and 1.3 ppm lipid peaks: 
\[
\% = \frac{A_{\text{water}}}{A_{\text{lipid}}} \times 100.
\]

The \([t\text{Cho}]\) measurement was expected to be independent of the voxel lipid fraction because all of the metabolites that contribute to the tCho peak are water-soluble. In voxels with low to moderate lipid content, \([t\text{Cho}]\) was independent of the lipid fraction. In voxels with large lipid content, however, the \([t\text{Cho}]\) measurement increases with increasing lipid fraction. Lipids apparently contribute to the amplitude of the tCho peak, either through baseline artifacts not suppressed by TE averaging or by a true resonance at 3.25 ppm. Because of this lipid contamination, we arbitrarily chose a cutoff value of 33%, above which the \([t\text{Cho}]\) measurement is considered biased and the MRS measurement is considered invalid.

Figure 5 shows a comparison between the internal and external reference schemes described in Materials and Methods. This shows that in good-quality spectra, where tCho is detectable and the lipid content is low, both internal and external methods produce consistent results (\(R^2 = 0.91\)). The absolute values produced by the external method are lower, due to overestimation of the product \(V \cdot \rho_{\text{water}}\) in the denominator of Eq. [10], which represents the aqueous content of the voxel.
The applicability of the \([t\text{Cho}]\) measurement for diagnosing different pathologies is shown in Fig. 6. All spectra that were of sufficient quality (i.e., free of artifacts, lipid fraction \(\leq 33\%\)) and were acquired from lesions with biopsy-confirmed pathology were divided into a “malignant” category (including infiltrative ductal, lobular, and unspecified carcinomas) and a “benign” category (including atypical hyperplasias, fibroadenomas, fibrocystic changes, and cysts). There were insufficient data to distinguish further histological subcategories or tumor-staging grades. When multiple spectra were acquired from a single lesion, only the spectrum with the smallest error was included in this chart. Spectra labeled “normal” were acquired from normal volunteers and from regions of normal-appearing and asymptomatic glandular tissue in other subjects. These results show that the \([t\text{Cho}]\) measurement is elevated in malignancies and some benign lesions. The mean \([t\text{Cho}]\) is greater in malignancy than in benign tissues \((P = 0.008, \text{one-tailed } t\text{-test})\), but the difference between the normal and benign categories is not statistically significant \((P = 0.17)\). An ROC analysis was performed to determine a threshold \([t\text{Cho}]\) value for distinguishing between benign and malignant lesions. Using equal weighting for false positives and false negatives, the criteria for malignancy is \([t\text{Cho}] \geq 1.38 \text{ mmol/kg}\). With this cutoff, the sensitivity is 46% and the specificity is 94%. Note that neither of these analyses take into account the variable sensitivity.

Single-voxel MRS in the breast is very sensitive to the size and placement of the voxel because of the heterogeneous distribution of tCho in the breast, as can be seen in the spectra shown in Fig. 7. All three spectra were acquired from different regions of the same 3-cm tumor, a grade III invasive ductal carcinoma that was studied after the patient had received 4 months of chemotherapy. In a large voxel covering most of the tumor, \([t\text{Cho}]\) was measured to be \(1.1 \pm 0.6 \text{ mmol/kg}\). The anterior, enhancing portion of the tumor had a higher \([t\text{Cho}]\) \((1.5 \pm 0.5 \text{ mmol/kg})\), whereas the posterior, non-enhancing region had no detectable peak \((|t\text{Cho}| < 0.5 \text{ mmol/kg})\). The sensitivity is lowest in the posterior voxel due to its distance from the coil, small voxel size, and higher lipid content. This example underscores the importance of proper voxel placement.

**DISCUSSION**

This work describes a new method for quantifying tCho levels in breast tissue. Quantitative MRS is a substantial improvement over the qualitative detection methods used in previous studies of breast MRS. Quantification is particularly important in the breast because the sensitivity of the MRS measurement is generally more variable than it is in brain tissue. This is due primarily to the highly variable adipose tissue content of the breast and the greater variation of the coil receive efficiency. Previous in vivo studies used the hypothesis that detectability of tCho is associated with malignancy; however, this approach is only valid if the detection threshold is constant. We have found that the use of a higher \(B_0\) field (4 T) and optimized surface coils increases the sensitivity enough to enable the detection of tCho in normal breast tissue and several benign lesions.

Two previous reports used external referencing to quantify choline levels in breast tissue (7,13). Neither of these studies corrected for partial volume of adipose tissue within the voxel. In our experience, the amount of intra-voxel adipose tissue can vary greatly, as shown in Fig. 4b. The breast can be coarsely approximated by a two-compartment model consisting of aqueous regions (fibroglandular tissue) and aliphatic regions (adipose tissue). The aqueous compartment contains all of the choline-containing compounds that are known to be elevated in malignancies. Any externally-referenced method must account for this compartmentation to avoid systematic error, whereas

![Fig. 5. A comparison between \([t\text{Cho}]\) calculated with both internal and external referencing methods in 98 spectra, all of which had detectable tCho and a lipid fraction of \(\leq 33\%\). A linear fit through the origin gives \(R^2 = 0.91\) and a slope of 0.72. This shows that these measures are highly correlated, although the external method produces somewhat smaller values.](image)

![Fig. 6. Measurements of \([t\text{Cho}]\) in malignant, benign, and normal tissues. Error bars represent SDs in spectra where tCho was detectable, and MDLs in spectra where no tCho was detectable. Measurements are ordered from the largest to the smallest in each category. Malignant and benign spectra were from biopsy-confirmed lesions only. Normal spectra were selected from healthy-appearing glandular tissue from subjects with no history of disease.](image)
an internally-referenced method compensates for compartmentation automatically. Internal referencing methods have further advantages in that they do not require separate calibration experiments, and they use fewer error-introducing correction factors for coil loading, coil efficiency, and voxel volume.

The disadvantage of the internal referencing approach is that it requires the density of NMR-visible water in the aqueous compartment to be relatively constant. There are physiological conditions that affect the water content, but these effects are expected to be small compared to the variability of compartmentation. Both internal and external approaches will be affected by the presence of edema from previous invasive procedures or radiation therapy, and by variations in cellularity and relaxation rates. Previous reports expressed concern that the relaxivity of gadolinium contrast agents would deleteriously affect MRS measurements (8). In our experience, gadolinium effects are small (~10%) compared to the measurement errors and variability of tCho levels (22).

The spectral fitting software used in this work is highly specialized for fitting peaks in breast spectra, and is available from the authors by request. The method is based on the TDFD technique, which uses an analytical TD model but minimizes residuals in the FD (14). We modified that technique by adding a linear baseline correction and using

FIG. 7. Spatial variation of [tCho] measurements within a single tumor. All spectra are from a 3-cm tumor of invasive ductal carcinoma after 4 months of chemotherapy treatment. Images on the left show voxel positions on a sagittal slice from a contrast-enhanced, fat-suppressed 3D FLASH image. Water-suppressed spectra on the right are shown with the tCho fit above and the residual (including baseline) underneath. In spectrum C, where no tCho was detectable, the MDL of tCho is shown with a dotted line. a: Voxel containing both enhancing and non-enhancing regions of the tumor, volume = 7.8 mL, [tCho] = 1.1 ± 0.6 mmol/kg, lipid fraction = 5%. b: Voxel acquired from the most enhancing region of the tumor, volume = 1.1 mL, [tCho] = 1.5 ± 0.5 mmol/kg, lipid fraction = 2%. c: Voxel acquired from a non-enhancing region, volume = 0.9 mL, [tCho] = 0 ± 1.3 mmol/kg, lipid fraction = 13%.
a very narrow frequency band (0.4 ppm) to fit a single peak at a time. By using only the real component of the spectrum, along with the narrow frequency band and the linear baseline correction, small tCho peaks can be fit without bias from large neighboring lipid resonances. Another useful feature of the TDFD method is that it permits the use of the Voigt lineshape, which can fit purely Lorentzian or purely Gaussian lineshapes, or any combination of the two. This flexibility was useful for this application because the use of a Lorentzian or Gaussian model alone did not produce good-quality fits for all peaks. The tCho peak is difficult to model precisely because it is a superposition of several resonances, and at 4 T there is not sufficient spectral resolution to separate the individual components. Also, imperfect correction of respiration-induced frequency shifts can cause “blurring” that leads to Gaussian lineshapes in averaged spectra. Although the Voigt model did not always produce an ideal fit (e.g., Fig. 2b), it performed reasonably well in most cases.

The TDFD method was chosen because it is well suited to the problem and is relatively easy to implement. There are numerous other methods that could be adapted for fitting breast spectra; however, most of these methods are incapable of fitting a precise frequency range or analytically modeling a non-Lorentzian lineshape (23, 24). The LCModel method (25) is very useful for fitting brain spectra, and it may also be applicable for fitting breast spectra. With the currently available implementation of the LCModel software, we were unable to get good-quality fits of small tCho peaks in spectra with large lipids. If the LCModel method could be adapted for breast spectra, it might offer some advantages, such as producing more accurate fits due to its sophisticated baseline model, and enabling the fitting of other metabolites that are occasionally observable in breast spectra, such as creatine, glycine, lactose, and taurine. Automated fitting methods are generally desirable because they eliminate user interaction and provide a well accepted measure of error (Cramer-Rao bounds) unbiased by peak lineshape. However, a simple analysis using peak integration and linear baseline correction over manually-defined extents gave comparable results (data not shown).

Investigators commonly use CRMVBs to estimate the variance of model parameters when fitting NMR spectra (21). In this work, the fitting error is used as an estimate of the overall measurement error, although it is probably an underestimate. Most notably, the CRMVB errors from fitting the water are very small, but the actual error in estimating the water amplitude is higher. This occurs because the Cramer-Rao theory assumes that the data are perfectly described by the model function, and this assumption is violated by irregular water lineshapes and the overlapping 5.4 ppm lipid resonance. Additionally, certain experimental factors (e.g., patient motion and respiration) can make the true measurement error greater than the fitting error. The true measurement error can only be established through repeatability studies. Nevertheless, some estimate of the measurement error is essential for any quantification scheme, and it should be incorporated into the interpretation of quantitative MRS results. It is particularly important in breast MRS because of its highly variable sensitivity, as demonstrated in Fig. 3a. Note that this sensitivity can be estimated prior to the acquisition of an entire spectrum by using the SNR of the water peak in a single-shot, unsuppressed spectrum. This information can be used to prescreen voxels for a diagnostic study.

The iterative MDL method described here (in which a simulated tCho peak is added to a spectrum and fit) can be used to measure the sensitivity in spectra with no detectable tCho peak. This is critical for interpreting whether a negative finding of tCho is due to lack of sensitivity or low tCho levels. The MDL is closely related to the fitting error: under ideal conditions, the fitting error is equal to the MDL, but in practice the MDL is often larger.

The use of a quantitative method to measure tCho levels in the breast increases the usefulness of MRS for diagnosing benign and malignant lesions. Figure 6 shows [tCho] measurements divided into three broad pathological categories. The finding that tCho levels are higher in malignancies than in benign or normal tissues is consistent with previously published reports that used the detection of tCho to indicate malignancy (7–11). The [tCho] threshold of 1.38 mmol/kg, based on an ROC analysis, can be used to distinguish between malignant and benign lesions; however, the sensitivity is poor (46%). More data must be acquired to determine whether subdividing the malignant and benign categories will improve these results (e.g., in the benign category, the two highest values were atypical hyperplasias). Note also that this ROC analysis is only a first-order method of interpreting the [tCho] levels. More sophisticated analyses should be used to account for the measurement errors and [tCho] probability distribution functions.

The absolute tCho levels (0.4–10 mmol/kg) reported in this work are reasonably consistent with previous in vivo estimates. Roebuck et al. (7) found choline levels of 0.4–5.8 mmol/L, and estimated their detection threshold was 0.2 mmol/L. Bakken et al. (13) reported a single measurement of 2 mmol/L. Further investigations are required to determine whether the absolute [tCho] values reported here are reproducible on different MR scanners and in other institutions.

Figure 6 shows a large range of [tCho] measurements in known cancers, from 0.5 to >8 mmol/kg. tCho levels did not appear to be related to different histological types of cancer (e.g., lobular vs. ductal); however, there were insufficient data to establish statistical significance. The variation in [tCho] was also not explained by complicating factors such as recent biopsy or previous chemotherapy treatments, although some of the spectra in which no tCho was detected were acquired from tumors that had been recently biopsied. The large range in [tCho] measurements may be a natural feature of cancer, due to variations in the amount of water, lipids, and stroma within the lesion and adjacent tissues, and to variations in intracellular tCho levels and neoplastic cell density. Previous chemotherapy, radiation, or invasive procedures may further complicate these issues. Additionally, certain experimental factors can contribute to inaccuracy in these measurements—most notably patient motion and incorrect voxel positioning. If either the biopsy needle or the MRS voxel is placed in the wrong region, there will be no agreement between the [tCho] measurements and the histology. Finally, it
should be noted that MRS measurements can be adversely affected by metallic radiographic markers, which are widely used but cannot always be identified in heterogeneous breast tissue.

CONCLUSIONS

In this work we report a new technique for quantifying tCho levels in breast cancer using single-voxel 1H MRS. With the use of optimized surface coils and a high-field (4 Tesla) scanner, we measured tCho levels in normal breast tissue and in benign and malignant lesions. The levels of tCho were found to be elevated in malignancies compared to benign lesions, indicating that quantitative MRS may be used to aid in diagnosing breast lesions and monitoring response to cancer treatments.

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