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BREAST IMAGING
ORIGINAL ARTICLE

Single voxel *in vivo* proton magnetic resonance spectroscopy of breast lesions: experience in 77 cases

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PURPOSE
We aimed to determine the value of in vivo single voxel proton magnetic resonance spectroscopy (MRS) in characterizing breast lesions.

MATERIALS AND METHODS
Breast MRS was performed in 77 patients. Choline resonance peak at 3.2 parts per million (ppm) was defined positive when it was at least two times higher than baseline. MRS findings were compared with the final diagnosis of cases for two different values (3.23 and 3.28 ppm).

RESULTS
Thirty-one malignant and 13 benign lesions had choline peaks. Sensitivity was 84%, specificity was 64%. Positive likelihood ratio (LHR) was 2.32, negative LHR was 0.25. Twenty-two malignant and 5 benign lesions had a peak at 3.23 ppm. Nine malignant and 8 benign lesions had a peak at 3.28 ppm. When 3.23 ppm was accepted as positive; sensitivity, specificity, and positive and negative LHRs were 79%, 82%, 4.4, and 0.26, respectively.

CONCLUSION
MRS provides additional parameters on evaluation of breast lesions. However, MRS of breast has some false negative results, thus it is still insufficient in clinical diagnosis.

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Traditional approaches for the assessment of breast lesions have limited sensitivity and specificity. Since mammography, ultrasonography (US), and contrast-enhanced magnetic resonance imaging (MRI) are unable to reliably distinguish between malignant and benign tissues, the final diagnosis of cancer is most often based on histopathological analysis.

Contrast-enhanced MRI can detect breast cancers with a high degree of sensitivity (ranging from 95% to 100%), but it also has an increased rate of false-positive enhancement of benign breast lesions. This results in a relatively low specificity that ranges from 37% to 97% and could contribute to increased rate of biopsy. Approximately 75% of the breast tumors detected by mammography and approximately 50% of contrast-enhanced lesions detected by MRI have benign histology (1). One method of for avoiding these deficiencies is magnetic resonance spectroscopy (MRS), which offers a noninvasive approach for differentiating malignant from benign lesions.

Proton (^{1}H) MRS is a noninvasive *in vivo* method that can be used to study metabolic changes in several pathologic conditions of different organs. The signal primarily comes from protons in water and lipids, and it is widely used in brain and prostate imaging. Although several metabolites, such as creatine, inositol, glucose N-acetyl aspartate, alanine and lactate, can be detected by MRS, the diagnostic value of MRS in breast imaging is typically based on the detection of elevated levels of choline compounds like phosphocholine (PC) and glycerophosphocholine (GPC) (2). In support of this, recent *ex vivo* MRS studies of different tumors have shown elevated levels of cholines (Ch), which can serve as tumor markers (3–5). The aim of this study was to determine the value of MRS in enhancing the specificity of the noninvasive analysis of potentially malignant breast lesions.

Materials and methods

The study was approved by the institutional review board of our institute. All participants gave written informed consent. Conventional breast MRI was performed with a 1.5 Tesla MRI device (Signa HDx, General Electric Healthcare, Milwaukee, Wisconsin, USA) and a dedicated eight-channel high definition breast coil. Seventy-seven patients were included in this study; patients had different indications for breast MRI following appropriate evaluation by mammography and/or US. All patients were examined in the prone position, and the breasts were slightly compressed from the lateral sides by compression paddles, taking care not to apply too much pressure on the tissue.

The routine sequences were axial short TI inversion recovery, sagittal fast spin echo, fat-saturated T2-weighted and sagittal three-dimensional VIBRANT (postcontrast fat-saturated T1-weighted gradient echo

sequence). For the dynamic series, two precontrast and six postcontrast series were carried out with a temporal resolution of about one minute (depending on the size of the breast and the number of images per sequence). In order to ensure examination of the enhanced lesion, MRS examination was performed following the dynamic contrast-enhanced sequence. The presence of gadolinium chelates is not thought to adversely affect the performance of breast MRS (6).

The area of investigation or region of interest was placed by one of two radiologists (S.O. or I.B.), taking care to encompass the lesion in the voxel. The voxel size, which ranged from 3 to 8 cm^3, was determined based on the size of the lesion, trying to exclude any fat tissue. Data was collected from a single rectangular volume of interest. The proton spectrum was collected with a BREASE sequence, a breast-specific, single-voxel spectroscopy application designed for ease-of-use and enhanced visualization (General Electric Healthcare). Repetition time was 2000 ms, echo time was 155 ms, number of excitations was 32, imaging time was 4 min and 48 s, and voxel thickness was 20 mm. Saturation bands on four sides of the voxel and automatic shimming were used.

The presence of a Cho resonance peak at 3.2 parts per million (ppm) was defined as positive when it was at least two times higher than baseline. All other cases were deemed to be negative. Then the spectra of the Cho positive cases were further classified into two groups of 3.23 ppm and 3.28 ppm, according to the location of the peak. The MRS results were compared with the final diagnosis of the cases. All the patients were evaluated according to Breast Imaging Reporting and Data System (BIRADS) classification, and the results were evaluated statistically using a computer software (Statistical Package for Social Sciences, version 16.0, SPSS Inc., Chicago, Illinois, USA).

Results

There was no significant difference between the ages of the patients in the malignant and benign lesion groups (range, mean, and median: 30–76 years, 47.5 years, 47.0 years for malignant group; 26–70 years, 45.5 years, 44 years for benign group; $P = 0.4$). Malignant lesions were significantly larger than benign ones (mean, 4.5 cm and range, 1–20 cm for malignant group; mean, 1.7 cm and range, 1–7 cm for benign group; $P = 0.001$).

In order to evaluate the performance of MRS; the sensitivity, specificity, positive predictive value (PPV), and positive and negative likelihood ratios (+LHR and –LHR) were calculated for two conditions. Initially, all cases with a Cho peak at 3.2 ppm were considered as positive; in further analysis, cases with a peak at 3.28 ppm were excluded and only the peaks at 3.23 ppm were considered positive. Histopathological analysis was carried out for all cases except 11 normal breast tissues and five BIRADS II lesions as defined by the BIRADS MR lexicon (one cyst and four fibroadenomas) (7). The results of the malignant and benign cases, their histopathological results, and the presence and location of the Cho peak are summarized in Table 1. Histopathological analysis revealed 40 (52%) of the 77 lesions to be malignant and 37 (48%) to be benign. Of the 40 malignant cases, 32 were invasive ductal carcinomas (Figs. 1 and 2), 1 was an invasive lobular carcinoma, 1 had a mix of invasive lobular and ductal features, 1 was a mucinous carcinoma, 1 was a ductal carcinoma in situ, and 1 was a diffuse leukemic infiltration of the breast (Fig. 3). The 37 benign cases consisted of normal breast tissue (11 cases), fibroadenomas (nine cases, Fig. 4), phyllodes tumor (two cases), fibrocystic changes (five cases), mastitis (five cases), postoperative hematoma (two cases), postoperative infection (one case), tuberculosis abscess (one case), and a simple cyst (one case). The 11 cases with spectral analysis of normal breast tissue showed no abnormalities by US, mammography, or MRI examination in the region of interest (BIRADS I). The simple cyst and three cases of patients receiving neoadjuvant chemotherapy (all showing disappearance of the Cho

Table 1. Distribution of 77 cases examined by proton MRS according to the final diagnosis, presence of Cho peak, and location of the resonance peak either at 3.23 or 3.28 ppm

Final diagnosis	n	Cho peak (–)	Cho peak (+)	Resonance peak at 3.23 ppm	Resonance peak at 3.28 ppm
Malignant lesions					
IDC	32	4	28	20	9
ILC	1	1	-	-	-
Mixed ILC+IDC	1	1	-	-	-
DCIS	1	-	1		
Mucinous carcinoma	1	0	1	1	
Leukemia	1	0	1	1	-
Postneoadjuvant chemotherapy	3	3[a]	-	-	-
Total	40	9	31	22	9
Benign lesions					
Normal breast tissue	11	9	2	1	1
Fibroadenoma	9	4	5	1	4
Filloides tumor	2	-	2	-	2
Mastitis	5	4	1	1	-
Fibrocystic changes	5	5	0		
Postoperative hematoma	2	1	1	1	-
Postoperative infection	1	-	1	-	1
Tuberculosis abscess	1	-	1	1	-
Cyst	1	1[a]	-	-	-
Total	37	24	13	5	8

DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.
[a]These cases were excluded from the statistical analysis.

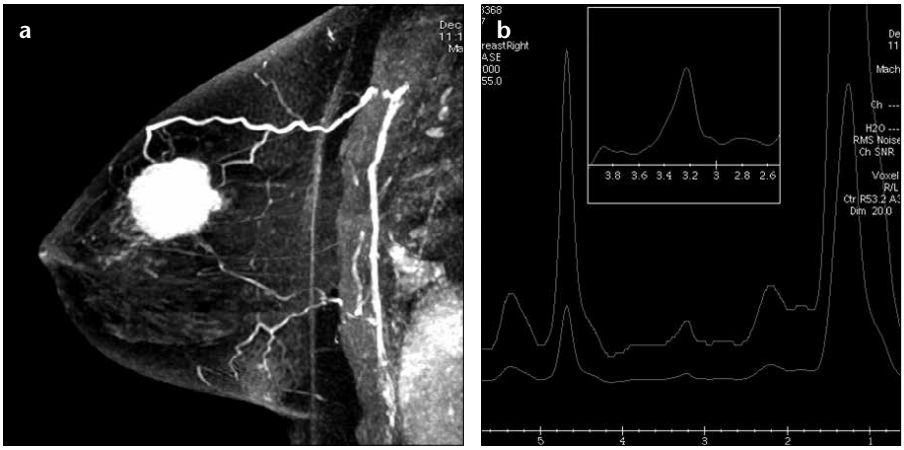

Figure 1. a, b. A 49-year-old woman with invasive ductal carcinoma. Sagittal MIP image **(a)** obtained from the postcontrast dynamic series demonstrates the enhanced mass lesion with irregular contours. MRS **(b)** shows a prominent Cho peak at 3.23 ppm, which is consistent with malignancy.

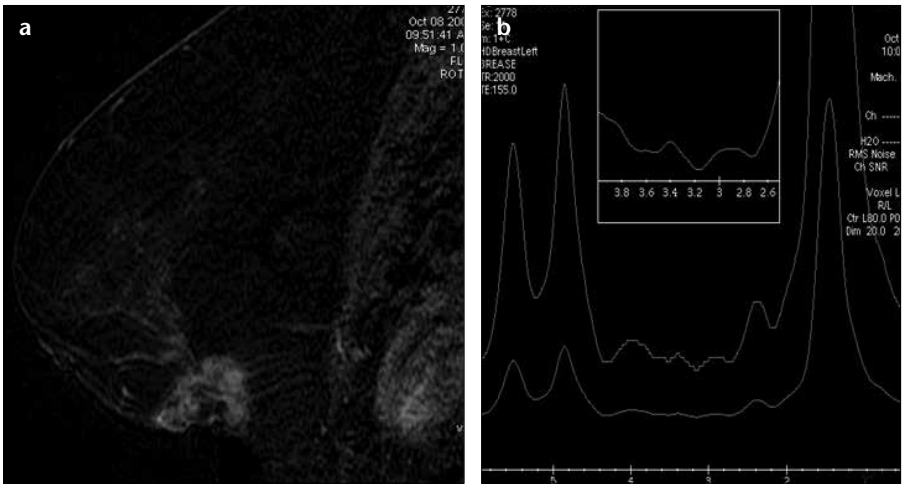

Figure 2. a, b. A 65-year-old woman with invasive ductal carcinoma. Postcontrast sagittal subtraction image **(a)** from the dynamic series demonstrates an ulcerated mass lesion infiltrating the skin at the inframammary sulcus. False-negative MRS is seen with no Cho peak **(b)**.

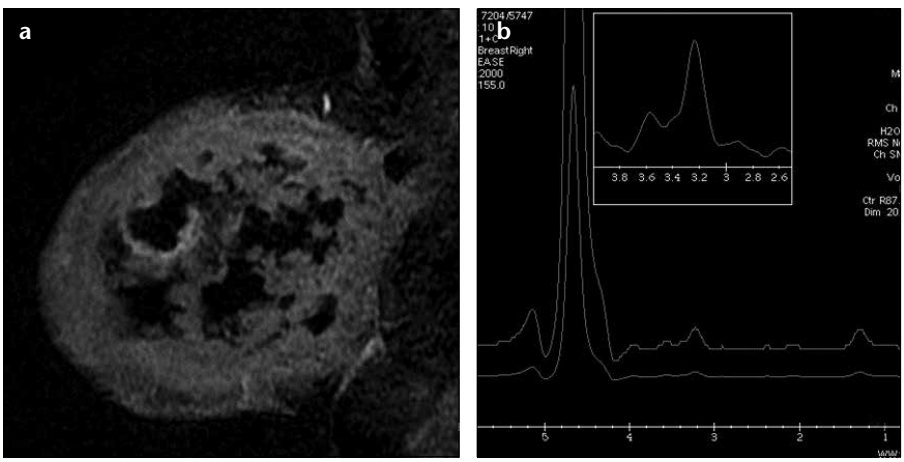

Figure 3. a, b. A 32-year-old woman with leukemic infiltration of the breast. Postcontrast sagittal subtraction image **(a)** from the dynamic series shows huge mass lesion with peripheral enhancement and central necrosis. High-amplitude Cho peak at 3.23 ppm is detected by single voxel MRS **(b)**.

peak, Fig. 5) were excluded from the statistical analysis. A Cho peak at 3.2 ppm was present in 31 of 37 malignant lesions and 13 of 36 benign lesions, giving MRS a sensitivity of 84% (95% confidence interval [CI], 67%–93%) and a specificity of 64% (95% confidence interval, 46%–79%). +LHR was 2.32 (95% CI, 1.47–3.66), and –LHR was 0.25 (95% CI, 0.12–0.54). PPV was calculated to be 70%.

When cases with a peak at 3.28 ppm were excluded and only cases with a peak at 3.23 were considered, 22 of 37 malignant and 5 of 36 benign lesions had a positive MRS. This resulted in a sensitivity of 79% (95% CI, 59%–91%) and a specificity of 82% (95% CI, 62%–93%). +LHR was 4.4 (95% CI, 1.94–9.96), and –LHR was 0.26 (95% CI, 0.13–0.54). PPV increased to 79% at this ppm value.

Discussion

In vivo proton (^{1}H) MRS, a powerful tool for exploring the cellular chemistry of human tissues, has previously been proposed as an adjunct method for breast MRI. MRS produces a graph of the resonance amplitudes of various metabolites on the y axis versus their resonance frequencies (in ppm) on the x axis. The resonance amplitudes are not absolute quantities but rather the relative concentrations of the metabolite chemical structures. In MRS, the signal primarily comes from protons in water (4.7 ppm) and lipids (0.9 ppm). However, several other metabolites of interest, including N-acetyl-aspartate (2.02 ppm), Cho (3.2 ppm), creatine (3.02 ppm), myo-inositol (mI) (3.57 ppm), and lactate (1.32 ppm) can also be detected. MRS has previously been shown to differentiate benign from malignant breast lesions through the detection of increased levels of composite Cho compounds (free Cho, PC, and GPC) (6, 8–16).

Cho is a biochemical marker for metabolism, indicating the rate of cellular membrane turnover and proliferation. Previous studies have reported increased Cho metabolism in breast cancer cells. In addition, several studies have shown that the broad composite resonance at 3.2 ppm, which includes contributions from Cho, PC, GPC, mI, and taurine, is a unique marker for malignancy. In our evaluation of the clinical utility of MRS, the presence of composite Cho compounds was used as an indicator of malignancy, whereas their

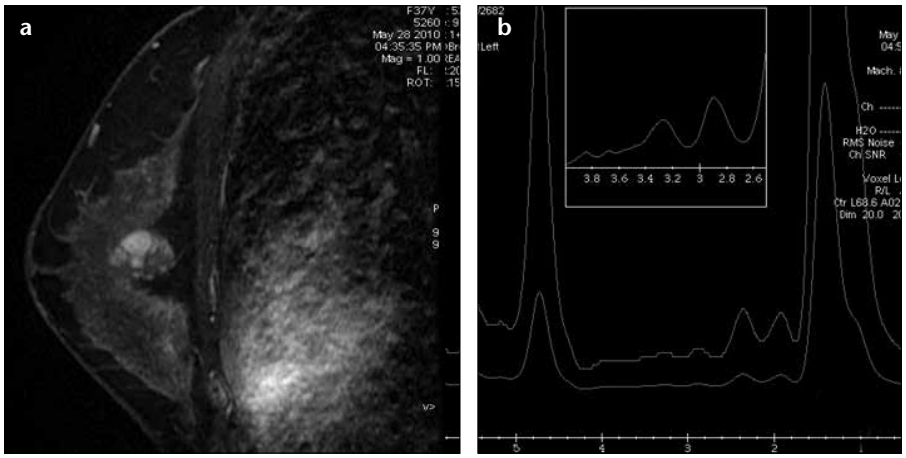

Figure 4. a, b. A 37-year-old woman with fibroadenoma. Sagittal fat-saturated postcontrast MRI image **(a)** shows a well-defined mass lesion with dark internal septa, indicative of a fibroadenoma. MRS **(b)** shows a Cho peak at 3.28 ppm, possibly representing GPC, mI, and taurine instead of Cho and PC.

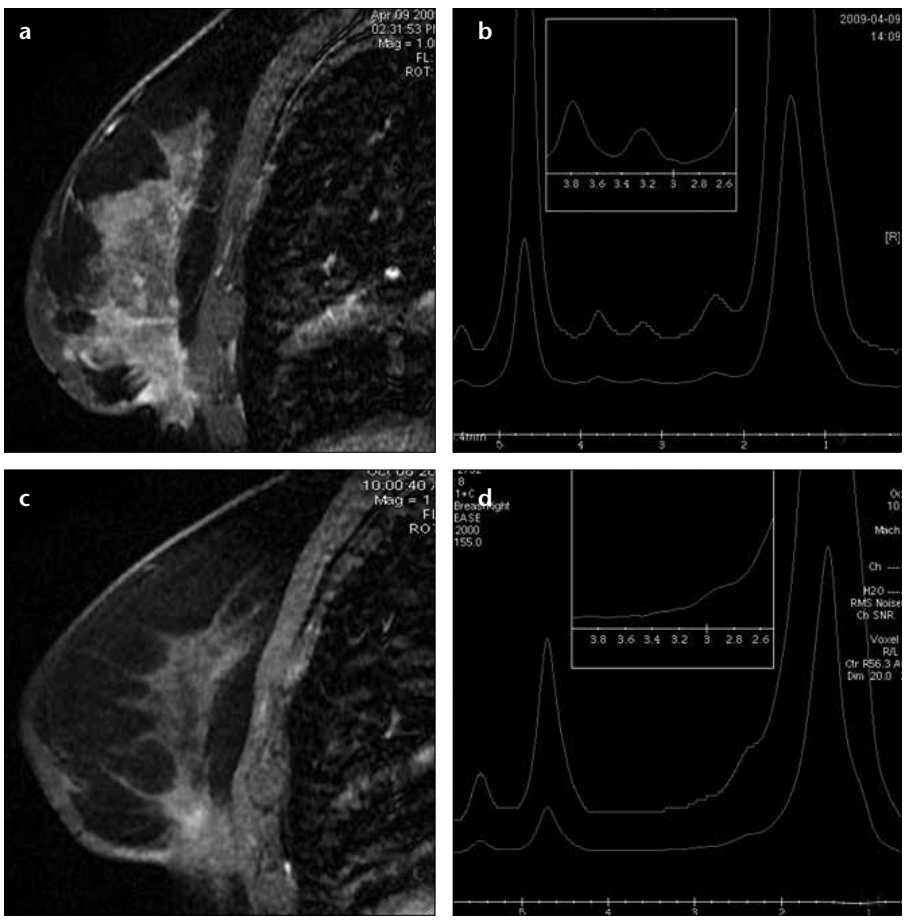

Figure 5. a–d. A 39-year-old woman with locally advanced mixed invasive ductal and lobular carcinoma. Prechemotherapy MRI **(a)** and MRS **(b)** show a Cho peak at 3.23 ppm. In the follow-up MRI **(c)** and MRS **(d)** after six cycles of neoadjuvant chemotherapy, the disappearance of the Cho peak and the decreased size of the mass lesion are noted.

absence indicated a benign lesion. This hypothesis is consistent with the 10-fold higher PC content that has been reported in human breast cancer cells relative to normal mammary epithelial cells (4, 17–19).

Previous studies have reported sensitivities of 70%–100% and specificities of 67%–100% for breast MRS (6, 8–16). The comparison of our results with those of previous studies is presented in Table 2; a total of 149 benign and 267 malignant cases have been examined by MRS. Relative to previous findings, we had a higher proportion of MRS examinations with false positive results, and therefore lower specificity and PPV, when the broad spectrum of 3.2 ppm was used as an indicator of malignancy. However, 8 of the 13 false positive benign lesions had negative results when the specific value of 3.23 ppm was used as an indicator of malignancy. As previously stated by Stanwell et al. (15), the breast carcinoma spectrum has a resonance at 3.23 ppm that is representative of PC whereas the fibroadenoma spectrum has a resonance at 3.28 ppm. This finding challenges the hypothesis that a malignancy may be confidently diagnosed on the basis of a positive broad composite Cho resonance at 3.2 ppm. We found that resolution of the composite choline resonance into its constituent components of 3.23 and 3.28 ppm improves the specificity of MRS. However, if we accept the presence of a resonance at 3.23 ppm as malignant and a resonance at 3.28 as benign, there is a higher risk of false negatives, which occurred in 19% of cases at this cut-off. Additionally, and in contrast with the 100% specificity for MRS reported by Huang et al. (13) and Bartella et al. (16), we had several false-negative results when using the composite 3.2 ppm resonance. Other studies on single-voxel MRS have also reported false-negative results (8–12, 14, 15); these were attributed to patient motion, adjacent air, or hemorrhage within the lesion that resulted in an inhomogeneous magnetic field.

Of the six false negative cases in this study, one was a case of invasive ductal carcinoma at the inframammary sulcus infiltrating the skin (Fig. 2) and two cases of invasive ductal carcinoma had postbiopsy MRS examinations. The heterogeneity of the tissue in these three cases may be the reason for their false negative MRS results. Two other cases were small T1 tumors (measuring 7 mm and 15 mm in diameter), which inevitably led to the inclusion of normal breast or fat tissue into the 20 mm voxel thickness. The sixth case was an invasive lobular carcinoma, in which the tumor cells tend to infiltrate as individual rows among the breast tissue and do not form a discrete mass

Table 2. Sensitivity, specificity, true positive (TP), true negative (TN), false negative (FN), false positive (FP), and positive predictive values (PPV) of previous studies in the literature in comparison to our study

Study	Number of malignant/benign cases	Sensitivity (%)	Specificity (%)	TP/TN (n/n)	FN/FP (n/n)	PPV (%)
Yeung et al. (6)	24/6	92	83	22/5	2/1	97
Roebuck et al. (8)	10/7	70	86	7/6	3/1	88
Kvistad et al. (9)	11/11	82	82	9/9	2/2	82
Cecil et al. (10)	23/15	83	87	19/13	4/2	90
Jagannathan et al. (11)	32/14	81	86	26/12	6/2	93
Tse et al. (12)	19/21	89	100	17/21	2/0	100
Huang et al. (13)	18/12	100	87	18/8	0/4	82
Kim et al. (14)	19/16	100	100	19/16	0/0	100
Stanwell et al. (15)	43/21	80	86	17/37	4/6	74
Bartella et al. (16)	31/26	100	88	31/23	0/3	91
Present study	37/36					
3.23 ppm		84	64	31/23	6/13	70
3.28 ppm		79	82	22/23	6/5	79

lesion. From these cases, it is clear that the sensitivity of MRS is a serious limiting factor preventing a wider adoption of this diagnostic tool for the management of breast lesions.

A homogeneous magnetic field and successful shimming are essential for sufficient water suppression and good spectrum quality. Close proximity to skin or inclusion of metalic clips, hemorrhage or fat tissue in the voxel results in inhomogenity and thus produces susceptibility effects. Due to the voxel thickness of 20 mm, small lesions cannot successfully be imaged by MRS. Currently, using a 1.5 T MRI system, *in vivo* MRS can only be performed with confidence on lesions larger than 1 cm^3 (20).

Two-dimensional spectra can overcome this problem by differentiating Cho groups from methylene protons that give rise to J-coupled multiplets in the 3–4 ppm region and by eliminating strong signal from adipose tissue mobile lipids that produce sidebands indistinguishable from other peaks (21). Further studies are necessary to evaluate the accuracy of MRS in breast lesions. Improvements that will enhance the utility of *in vivo* proton MRS include increasing the signal to noise ratio, generating consistent high spectral quality with high resolution and quantifying the resonance peaks. Furthermore, correlating spectral findings with the amount and type of contrast enhancement detected via MRI may lend support to the idea that adding spectral examination as an adjunct to routine breast MRI improves the diagnostic value of breast MRI. Additional studies comparing the spectral findings with histological and nuclear grades as well as tumor proliferation markers in a larger and more homogeneous group of patients may demonstrate the value of spectral analysis as a predictive and prognostic factor.

In conclusion, MRS is a promising technique that can be readily incorporated into a breast MRI examination and is rapid, noninvasive, and well tolerated. Single voxel spectroscopy cannot aid in lesion detection, but it can help to characterize suspicious lesions. Two-dimensional spectroscopic imaging will allow mapping of the spatial distribution of disease. In the future, higher-field-strength magnets and improvements in radiofrequency coil designs will improve the spectral resolution and signal-to-noise ratio, thus enabling the rapid examination of smaller lesions. However, more studies are needed to assess the added value of MRS in the diagnosis of malignant breast lesions. We propose that the ultimate goal should be to use MRS as a screening tool to detect slight increases in Cho in premalignant lesions without using contrast material.

Conflict of interest disclosure

The authors declared no conflicts of interest.

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