Breast cancer causes more than 40,000 deaths in women in the United States each year (1). Standard screening modalities for breast cancer are too often imprecise. Mammograms and MR images lack specificity because they present considerable overlap in the appearances of benign and malignant breast tissue (2). Recently, contrast material–enhanced breast MRI has enabled greater sensitivity for the detection of cancer than that of conventional MRI because it provides physiologic measures in addition to morphologic information. Contrast-enhanced breast MRI has proven to be a more sensitive technique for the detection and diagnosis of invasive breast cancer, and it is increasingly being used as an adjunct to mammography (2–5), and diagnosis of invasive breast cancer, and it is increasingly being used as an adjunct to mammography (2–5), although enhanced specificity is still needed.

In addition, in vivo MR spectroscopy at a high field strength has enabled scientists and clinicians to investigate the phospholipid biochemistry in breast tissue and the microenvironment of breast cancer. Both functional and metabolic information can be obtained and offer the opportunity to better differentiate benign from malignant tissue. By using the in vivo hydrogen 1 (1H) MR spectroscopic approach, it has been shown that total choline (tCho) is detected at higher concentrations in breast cancer tissue than in healthy breast tissue (6,7). MR spectroscopy at a high field strength can augment mammography and MRI by detection of individual molecular constituents of breast tumors (6,8). Knowing the specific molecular profiles of cancerous versus benign tumors could make MR spectroscopy a useful adjunct for differentiating between benign lesions and various types and grades of cancers. The tCho is the sum of multiple phosphatidylcholine (PtC) metabolites (primarily choline [Cho], phosphocholine [PC], and glycerophosphocholine [GPC]), which are related to cell-membrane and/or PtC metabolism. The latter two of these compounds are centrally involved in PtC metabolism because PC and GPC are the predominant membrane precursor and breakdown product, respectively.

Early MRI studies of preparations of malignant cells suggest that phospholipid metabolism is altered in tumors (9) and that PC is elevated in breast cancer (10). Furthermore, Glunde et al (11) found a change in the GPC/PC ratio with immortalization of mammary epithelial cells and malignant progression. These findings suggest that the relative levels of PC, GPC, and Cho may be indicators of the presence of specific breast cancers and the degree of malignancy. The metabolic profile of these metabolites has been associated with differences in gene expression for different cancer types (12). However, at the current level of MR technology, in vivo at 3 T, the resonances of these Cho-containing metabolites cannot be resolved from each...
Abbreviations

Cho = choline, FNA = fine-needle aspiration, GPC = glycerophosphocholine, IDC = invasive ductal carcinoma, PC = phosphocholine, PtC = phosphatidylcholine, tCho = total Cho, tCR = total creatine

Summary

Sixty percent of fine-needle aspiration (FNA) biopsy specimens yielded no usable hydrogen 1 MR spectra, and results were largely inconclusive when derived from usable spectra of histologically confirmed biopsy specimens; thus, the combined MR spectroscopic and FNA technique provided little value for detection and diagnosis of breast cancer.

Key Points

- Previous research has indicated that the relative levels of phosphocholine (PC), glycerophosphocholine (GPC), and total choline (tCho) may be indicators of the presence of specific breast cancers and degree of malignancy.
- Quantification of the relative levels of choline (Cho), PC, and GPC in fine-needle aspiration biopsy specimens from benign breast lesions, as well as a range of several histologically confirmed breast cancer types and grades, were assessed by using MR spectroscopy to determine whether the metabolic signatures could be used to elucidate the pathophysiologic mechanisms of distinct tumor types and to discriminate the degree of malignancy.
- PC, GPC, and Cho were measured relative to each other as well as to tCho; there was only a difference between PC to total creatine in the distinction between benign and malignant breast lesions ($P = .028$).

In Vivo MRI Procedure

At the time of MRI, each participant underwent contrast-enhanced dynamic 3-T breast MRI of both breasts, in which a standard clinical protocol was used. The resulting images were evaluated for staging and extent of disease of the presumed malignancy. MRI was performed by using a GE HDX 3-T whole-body MRI system (GE, Milwaukee, Wis). A phased-array 16-channel dual-breast coil was used to acquire both localized MR images.

Biopsy Procedure

Following MRI, each patient underwent image-guided biopsy of the suspicious breast mass. Biopsy of any other suspicious lesions identified at MRI and biopsy of any abnormal axillary lymph nodes identified at the time of original mammographic diagnostic work-up were performed at this time. The biopsy was scheduled after MRI, so as to avoid any blood-component contaminants. The biopsy may have taken place on the same day as MRI or at a later time, dependent on patient preferences, tolerance, and convenience. At the time of biopsy, the lesion was identified using the guidance method that best depicts the lesion, in accordance with standard clinical practice. For most cases, US guidance was used. If the lesion was not sonographically visible, stereotactic biopsy was performed. In the case of lesions identified only at MRI, MR-guided biopsy was performed. The correlation between the biopsy lesion and the lesion identified at MRI is routinely accomplished as part of standard clinical practice. Axillary lymph-node biopsies, if necessary, were performed with US guidance. On identifying the index breast lesion, each patient’s overlying skin was prepared in the standard clinical fashion and by using topical anesthetic because of the spectral proximity of the 1H MR spectroscopic signal of lidocaine to those of Cho-containing compounds (14). The FNA was performed first using a 22-gauge needle, followed by a large core-automated or vacuum-assisted biopsy, per standard of care. The FNA specimen was processed as described in the following section. The core specimens were transported to the pathology department for histopathologic
evaluation, which determined the diagnosis of the lesion to be benign, invasive ductal carcinoma (IDC), invasive lobular carcinoma, or ductal carcinoma in situ. IDC lesions were also be benign, invasive ductal carcinoma (IDC), invasive lobular carcinoma.

FNA Sample Preparation and MR Spectroscopic Data Acquisition

Biopsy samples were placed in a microtube containing 250 µL of phosphate-buffered saline in deuterium at a pH of 7.3, immediately frozen in powdered dry ice (−78.5°C), and stored at −80°C for no longer than 6 months. The individual (J.A.D., with 6 years of experience) who acquired the 1H MR spectra was blinded to the histopathologic results for the entire study and did not perform the later statistical analysis. Prior to MR spectroscopic data acquisition, samples were removed from storage, thawed, and mixed with 2 µL of a 0.00 ppm chemical-shift reference (10 mmol/L 2,2,3,3-d4 sodium 3-trimethylsilyl pro-
pionate), along with approximately 50 µL of phosphate-buffered saline in deuterium at a pH of 7.3 to achieve a total sample volume of approximately 300 µL. The solution was then transferred to a 5-mm restricted-volume susceptibility-matched MR spectroscopic tube (Shigemi, Allison Park, Pa). A susceptibility-matched plug was inserted and secured in place by using a sealing film (Parafilm M; Bemis Flexible Packaging, Neenah, Wis) after removing any air bubbles. High-spatial-resolution 1H MR spectra were acquired with a 400-MHz narrow-bore spectrometer (Varian Inova; Varian, Palo Alto, Calif) by using a triple-tuned 1H–carbon 13 (13C)–nitrogen 15 (15N) inverse probe. Second-order gradient shimming was performed on the deuterium signal by using an automated protocol, followed by frequency and power calibration for water presaturation. Imaging parameters for data acquisition were as follows: 4.7-second repetition time, 90° flip angle, 6-kHz spectral width, 16,384 complex points, and 256 averages, for a total imaging time of just over 20 minutes. The time between sample thawing and completion of data acquisition was less than 60 minutes. Some samples were spiked with pure standard compounds such as taurine, myo-inositol, and phosphoethanolamine to confirm PtC-metabolite peak assignments, which were consistent with those from the prior literature, and to assess the role of potentially interfering compounds (14).

Analysis of MR Spectra

High-spatial-resolution 1H MR spectra from FNA samples were analyzed individually off-line. Quantitation was performed by using version 5.2 of the jMRUI software package (www.jmru.eu). Major baseline distortions and large undesirable peaks (ie, from lipids or contamination with US gel and/or local anesthetic) were removed using a Hankel-Lanczos singular-value decomposition filter. The spectral region of interest from 4.0 to 2.5 ppm was isolated using jMRUI’s frequency-selective ER filter. Prior knowledge of chemical shifts and couplings (if applicable) was input for the signals of interest (Cho, PC, GPC, and tCr), as well as for the other previously mentioned compounds. The AMARES (advanced method for accurate, robust, and efficient spectral fitting) algorithm was used to determine the least-squares best fit of Lorentzian line shapes to the specified resonance signals (18). In this manner, the chemical-shift values and signal amplitudes were quantified for Cho, PC, GPC, and tCr. The determination of absolute metabolite concentration was not feasible because the masses of the FNA samples could not be reliably obtained. Accordingly, results were reported as ratios of individual or summed PtC metabolites to each other (Table 2) and to tCr (Table 3).

### Table 1: Number of Samples for Various Groups

| Group | All | Usable Spectra | Unusable Spectra |
|-------|-----|----------------|------------------|
| Benign | 55  | 10*            | 45               |
| DCIS  | 4   | 4              | 0                |
| IDC-1 | 10  | 5              | 5                |
| IDC-2 | 20  | 13†            | 7                |
| IDC-3 | 21  | 14†            | 7                |
| ILC   | 5   | 0              | 5                |
| Total | 115 | 46             | 69               |

Note.—Numbers hyphenated to IDC indicate grades. DCIS = ductal carcinoma in situ, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma. *Five spectra displayed no total creatine and two displayed no choline. †Two spectra displayed no total creatine. ‡One spectrum displayed no total creatine.

### Table 2: Mean PtC-Metabolite Ratios Relative to Each Other and to tCho for FNA Samples from Benign Lesions and Various Cancer Lesions

| Group | PC/GPC* | Cho/GPC | PC/tCho† | GPC/tCho‡ | Cho/tCho |
|-------|---------|---------|----------|-----------|----------|
| Benign| 1.74(9.00) | 0.29(1.71) | 0.49(0.05) | 0.40(0.05) | 0.10(0.03) |
| DCIS  | 4.07(14.2) | 0.42(2.70) | 0.69(0.08) | 0.24(0.07) | 0.06(0.05) |
| IDC-1 | 2.04(12.7) | 0.25(2.41) | 0.58(0.07) | 0.35(0.07) | 0.07(0.04) |
| IDC-2 | 15.8(7.89) | 2.99(1.50) | 0.51(0.05) | 0.40(0.04) | 0.10(0.03) |
| IDC-3 | 2.97(7.61) | 0.58(1.44) | 0.58(0.04) | 0.29(0.04) | 0.13(0.02) |

Note.—Metabolic ratios with standard errors in parentheses are shown. Numbers hyphenated to IDC indicate grades. tCho consists of PC, GPC, and Cho. Cho = choline, DCIS = ductal carcinoma in situ, GPC = glycerophosphocholine, IDC = invasive ductal carcinoma, PC = phosphocholine, PtC = phosphatidylcholine, tCho = total Cho. *Benign group different from total cancer groups at P = .076. †DCIS group different from total IDC groups at P = .065. ‡Benign group different from total cancer groups at P = .099.
Statistical Analysis
Statistical testing was performed by using SAS software version 9.4 (SAS Institute, Cary, NC). The total malignant group was compared with the benign group by planned \( t \) test contrasts for all MR measures. Within the total malignant group, the ductal carcinoma in situ group was compared with the mean of the three IDC groups by independent-sample \( t \) test contrast. The IDC groups were examined for linear trends (ie, based on linear contrast coding among the three IDC groups, in which the contrast is a test of whether the mean responses significantly correlate with the ordered category labels \([-1, 0, 1]\)). Comparisons were regarded as significant when the two-sided \( P \) value was less than .05. Given our modest sample size, we determined that to maintain a reasonable balance between potential for false discovery of associations (type 1 error) and potential for missed discovery of genuine associations (type 2 error), we would not adjust individual hypothesis tests for multiple testing.

We performed an additional set of exploratory multivariate analyses to determine whether joint information from multiple metabolite ratios might improve prediction of lesion type above the information provided by univariate analysis. Using stepwise logistic regression of each lesion type on the set of metabolite ratios (with an inclusion threshold of \( P < .05 \) and a deletion threshold of \( P > .10 \) at each step), we did not observe significant incremental benefit of using joint predictors (ie, in each case, the selected model included no more than one ratio).

Results

MR Spectra of an FNA Biopsy Specimen
The Figure shows the pertinent 3.0- to 3.5-ppm region of a typical MR spectrum of an FNA biopsy specimen from a malignant lesion. The FNA spectra show the tCho peak to be partially resolved into its components GPC, PC, and Cho, with little overlap with taurine. The individual Cho-containing metabolites are readily quantified relative to each other or to tCr, when detected.

Assessment of MR Spectra Quality
A total of 69 samples were ultimately excluded from the analysis because they yielded spectra with no observable resonances (presumably because of an insufficient number of cells aspirated) or suffered from uncorrectable spectral interference owing to large lipid signals. As provided in Table 1, 46 samples, which included no invasive lobular carcinoma samples, yielded analyzable spectra similar to those demonstrated in the Figure. In a small number of cases (Table 1), because no tCr or Cho signal was observed in an otherwise usable spectrum, that spectrum was excluded from the affected analyses.

Metabolite Comparison between Benign and Malignant Breast Lesions
Table 2 presents the means and standard errors of ratios of individual and summed PtC metabolites to tCr, whereas Table 3 presents ratios of individual and summed PtC metabolites to tCr. For the results in Table 2, no statistically significant \( (P < .05) \) differences were found between groups. However, trends were noted for PC/GPC \( (P = .076) \) and GPC/tCho \( (P = .099) \) for the comparison of the benign and total cancer groups and between the ductal carcinoma in situ and total IDC groups \( (P = .065) \). No linear trend was detected among the cancer groups. For the results in Table 3, only the difference in PC/tCr for the benign and total malignant groups was statistically significant \( (P = .028) \).

Discussion
Three methodologic changes relative to the prior study (14) were introduced in this study. First, the use of a topical anesthetic avoided the severe spectral interference often encountered with injected lidocaine in the initial study (14). The spectrum of the Figure is typical of spectra from the 46 samples for which usable data were obtained. Second, blinded preparation and acquisition of the MR spectra, with their automated analysis performed by using the widely accepted jMRUI software, eliminated the possibility of the potential user bias of the previous study (14). Third, the use of the tCr resonance as a possible internal standard was added to the measurement of ratios of the PtC metabolites themselves. In vivo MR spectroscopic studies have often used ratios of metabolites to tCr, which, by implication, are assumed to be relatively constant. Although the only statistically significant ratio difference in this study was the PC/tCr difference, no tCr was observed in eight of the 46 analyzable spectra, which provides a significant limitation to use of tCr as a standard.

Overall, the results of this study, as they relate both to the differentiation of benign and cancerous lesions and to the discrimination of various cancer types and grades, are disappointing. Our findings are not consistent with previous FNA studies of breast cancer achieving high sensitivity and specificity in distinguishing malignant tissue from benign tissue (16,17). Usable MR spectra were not obtained from 60% (69 of 115) of examined samples.
This result might have been useful had the overwhelming majority of the 69 samples been benign. Unfortunately, over 34% (24 of 69) of the unusable spectra were from samples of histologically confirmed cancer. We attribute this result to the inadequacy of FNA tissue sampling of diffuse and heterogeneous lesions, relative to the more reliable core biopsy. We conclude that the cells examined at MR spectroscopy of FNA biopsy specimens were often aspirated from a slightly different region that did not correspond to the salient characteristics of the breast lesion.

Our results weakly confirm that PC is the primary metabolite that increases in cases of malignancy. Interestingly, we did not find an increase in tCho/tCr for benign samples relative to cancer samples or for increasing IDC grade (except relative to IDC-1, for which only five samples provided usable spectra). Such might have been expected, given that increased tCho is usually observed by using in vivo MR spectroscopy for breast cancer.

With further study and improved sampling, the resolution of resonances from the separate Cho-containing compounds afforded by our methods may provide additional information about differences in cancer metabolism across different cancer types and grades. In future studies, these metabolic features could be used in the long-term evaluation of patients with breast cancer and to determine the molecular changes that occur with treatment. Through increased understanding of the mechanisms that cause the molecular changes seen in breast cancer, targeted therapies could be developed to prevent these changes from occurring altogether.

A limitation to our study and to the technique described was that the determination of cell type was established through histologic examination of the specimen from the core biopsy performed within the same procedure but performed separately from the FNA biopsy. There remains the potential that the cells examined by using MR spectroscopy were aspirated from a different area that did not correspond to the tissue analyzed by using core biopsy.

To summarize, given that 60% of FNA biopsy specimens yielded no usable 1H MR spectrum and that results were largely negative from usable spectra of histologically confirmed biopsy specimens, we conclude that the technique, as here modified and implemented by us, is of little value for detection and diagnosis of breast cancer. We recommend that a future study improve the way the FNA samples are acquired or use high-spatial-resolution proton magic-angle spinning, which enables the same measurements to be conducted on a core biopsy specimen while leaving the sample intact for further histologic analysis (12). In this manner, the MR spectroscopic data will necessarily correspond to the histologic results. An additional advantage of this method is a markedly increased spectral quality resulting from the greater amount of tissue used in the sample.

Figure: Typical spectrum of a fine-needle aspirate. The accompanying biopsy specimen was positive for grade 2 invasive ductal carcinoma. The pseudotriplets centered at about 3.27 and 3.44 ppm are from taurine. a.u. = arbitrary units, Cho = choline, GPC = glycerophosphocholine, PC = phosphocholine, tCr = total creatine.

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