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Quantitative In Vivo Proton MR Spectroscopic Assessment of Lipid Metabolism: Value for Breast Cancer Diagnosis and Prognosis

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Abstract

Background—Breast magnetic resonance spectroscopy (¹H-MRS) has been largely based on choline metabolites; however, other relevant metabolites can be detected and monitored.

Purpose—To investigate whether lipid metabolite concentrations detected with ¹H-MRS can be used for the non-invasive differentiation of benign and malignant breast tumors, differentiation among molecular breast cancer subtypes, and prediction of long-term survival outcomes.

Study Type—Retrospective

Subjects—168 women, aged 18 years.

Field Strength/Sequence—Dynamic contrast-enhanced MRI at 1.5T: sagittal 3D spoiled gradient recalled sequence with fat saturation, flip angle=10°, TR/TE=7.4/4.2, slice thickness=3.0

mm, FOV=20 cm, and matrix size=256×192. ¹H-MRS: PRESS with TR/TE=2000/135 ms, water suppression, and 128 scan averages, in addition to 16 reference scans without water suppression.

Assessment—MRS quantitative analysis of lipid resonances using LCModel was performed. Histopathology was the reference standard.

Statistical Tests—Categorical data were described using absolute numbers and percentages. For metric data, means (plus 95% CI) and standard deviations as well as median, minimum, and maximum were calculated. Due to skewed data, the latter were more adequate; unpaired Mann–Whitney U-Tests were performed to compare groups without and with Bonferroni correction. ROC analyses were also performed.

Results—There were 111 malignant and 57 benign lesions. Mean voxel size was 4.4±4.6 cm³. Six lipid metabolite peaks were quantified: L09, L13+L16, L21+L23, L28, L41+L43 and L52+L53. Malignant lesions showed lower L09, L21+L23, and L52+L53 than benign lesions (p=0.022, 0.027, and 0.0006). Similar results were observed for Luminal A or Luminal A/B vs. other molecular subtypes. At follow up, patients were split into two groups based on median values for the 6 peaks; recurrence-free survival was significantly different between groups for L09, L21+L23, and L28 (p=0.0173, 0.0024, and 0.0045).

Data Conclusion—Quantitative *in vivo* ¹H-MRS assessment of lipid metabolism may provide an additional non-invasive imaging biomarker to guide therapeutic decisions in breast cancer.

Keywords

Breast cancer; magnetic resonance spectroscopy; lipid; metabolism; radiometabolomics

Introduction

Medicine has entered the genomic era where precision treatment approaches requiring a deeper understanding of tumor biology and underlying oncogenic processes (1) are being explored and implemented in patient care (2). Reprogramming of cellular metabolism is a recognized cancer hallmark; the dysregulation of fatty acid and lipid metabolism is a quintessential component of malignant transformation in many cancers, including the breast. The differences in lipid metabolism of normal and malignant tissues make these pathways attractive targets for breast cancer diagnostics and therapeutics (3–5). In breast cancer, MRI has emerged as a powerful imaging technique that, in addition to morphologic changes, can simultaneously assess a plethora of functional, cancer-related processes (6–8). In addition, in recent years, several studies have demonstrated the potential of *in vivo* assessment of tumor metabolism with proton magnetic resonance spectroscopy (¹H-MRS) as a supplement to dynamic contrast-enhanced MRI for an improved diagnosis and characterization of breast tumor.

To date, in breast imaging, the value of MRS has been largely based on the detection of choline (Cho) metabolites for breast cancer diagnosis (9–16) and the spatio-longitudinal monitoring of changes in Cho metabolites during neoadjuvant chemotherapy (17–22). However, in addition to Cho metabolites, other relevant metabolites including lipids can be detected and monitored with ¹H-MRS. Initial *ex vivo* nuclear magnetic resonance and *in-*

vivo MRS studies demonstrated differences in fat and water between malignant, benign and normal tissue, showing that cancerous tissue display higher water concentrations and lower methylene lipid peaks at 1.3 ppm (21,23–27).

So far, although most of the research on lipid metabolites has focused on the detection of the 1.3 ppm lipid peaks from *in vivo* MRS, a whole spectrum of 9–10 lipid peaks with different chemical shifts can also be detected with MRS (28). A few studies have looked at L21, L23, and L28 resonances in the adipose tissue in breast controls (29) and contralateral breast adipose tissue in breast cancer patients (8). Thus, we hypothesized that through the non-invasive quantitative assessment of lipid metabolism with ¹H-MRS, insights into tumor biology are feasible which might be useful for the breast cancer diagnosis, characterization and prognosis. The purpose of this study was to investigate whether lipid metabolite concentrations detected with ¹H-MRS can be used for the non-invasive differentiation of benign and malignant breast tumors, for differentiation among molecular breast cancer subtypes, and for prediction of long-term survival outcomes.

Materials and Methods

As part of a health insurance portability and accountability act-compliant and institutional review board-approved prospective single-center study, patients gave written informed consent. A total of 177 patients were enrolled in this study. Nine patients were excluded due to non-evaluable spectral quality. Hence, for this study, 168 patients were retrospectively analyzed from the prospective database. Some patients (n=88) included in this study were previously analyzed with different software and reported in a different context, i.e., value of choline as a biomarker in breast cancer (21).

Patients

All 168 patients (median age: 48 years; range 26–75 years) underwent multiparametric MRI of the breast with T2-weighted, dynamic contrast-enhanced MRI and ¹H-MRS at 1.5 T for assessment of imaging abnormality or staging of breast cancer. Inclusion criteria were: age 18 years; imaging abnormality on mammography or sonography (asymmetric density, architectural distortion, suspicious microcalcifications or breast mass); lesion size 1 cm in diameter on dynamic contrast-enhanced MRI; and histopathologic verification. Exclusion criteria were: pregnancy, lactation, unable to undergo or complete a breast MRI examination, presence of a breast hematoma adjacent to the suspicious lesion due to intervention, prior neoadjuvant chemotherapy, and contraindication to MRI or contrast agents.

MRI and Single-voxel ¹H-MRS

All patients with breast lesions were examined with a 1.5T GE LX or Excite scanner (General Electric Medical Systems, Milwaukee, WI) with the body coil as the transmitter and a dedicated four-channel or eight-channel phased arrayed breast coil (InVivo Corporation, Orlando, FL) as the receiver. Dynamic contrast-enhanced MRI (sagittal 3D spoiled gradient recalled sequence with fat saturation, 10° flip angle, 4.2/7.4 ms TE/TR, 3.0 mm slice thickness, 20 cm field of view, and 256×192 matrix size) was performed in accordance to international recommendations (30,31).

Single-voxel proton MR spectral data covering the lesions were collected after recording dynamic contrast-enhanced MRI. Post-contrast sagittal T1-weighted images after the 1st time point at 60 sec were used as scouts for the placement of the MRS voxel to encompass the enhancing lesion.

Proton MRS acquisition was carried out using a point resolved spectroscopy sequence (PRESS) with TE of 135 ms, TR of 2000 ms, water suppression, and 128 scan averages, in addition to 16 reference scans without water suppression. A total of 128 scans were collected to be able to detect choline metabolite with higher signal-to-noise (SNR) using water signal for choline quantification. As our goal in the present study was to quantify the lipid metabolites, we were able to use the same data with water suppression for choline detection. Although we did not quantify the effect of gadolinium in terms of line broadening, the automatic shimming routine on the un-suppressed water peak during pre-scan usually achieved a full-width-at-half-maximum of less than 20 Hz (21). The total scanning time for the spectroscopy examination, including the pre-scan adjustment for shimming and water suppression, was less than 10 minutes.

Additionally, to demonstrate the improvement in lipid peak separation at higher field strengths (3.0 T), representative MR spectra was determined from a normal volunteer (age= 33 years) by covering an MRS volume within the glandular tissue ($1.5 \times 1.5 \times 1.5 \text{ cm}^3$). Stimulated echo acquisition mode (STEAM) imaging sequence (TR/TE=1500/14 ms and 64 scans) was employed to collect the spectral data using 3T MR750 scanner (GE Healthcare, Waukesha, WI) using an 8-channel breast coil.

¹H-MRS Data Analysis

MR spectroscopic analysis was performed using LCModel software (version 6.2, <http://sprovencher.com/pages/lcmodel.shtml>). This software deconvolutes the experimental spectra into a linear combination of each spectral peak within the MR spectra. We used the spectral range of -1 to 8 ppm using the SPTYPE= '8-breast' basis set in the control file. At 1.5T field strength, due to limited spectral resolution, some lipid peaks can overlap. Hence, we classified all lipid metabolites into six groups: L09 (0.9 ppm), L13+L16 (1.3 and 1.6 ppm), L21+L23 (2.1 and 2.3 ppm), L28 (2.8 ppm), L41+L43 (4.1 and 4.3 ppm), and L52+L53 (5.2 and 5.3 ppm), normalized by MRS voxel sizes (Figure 1). Lipid peaks were treated as positive/detectable when the Cramer-Rao lower bound > 30%.

Histopathological Analysis

In all patients, histopathology was used as the standard of reference. Histopathologic diagnosis was verified by a pathologist with more than 15 years of experience, using either image-guided needle biopsy or surgery (32). In the case of a benign histopathologic diagnosis at image-guided needle biopsy, the final diagnosis was benign (n=30). In the case of a high-risk lesion with uncertain potential for malignancy, the final diagnosis was established with open surgery (n=10) (33).

For malignant lesions, pathology reports were reviewed for tumor histologic type and histological and nuclear grades. Hematoxylin and eosin stained slides for the study were obtained from surgical pathology files. Tumors were classified using the World Health

Organization classification and invasive carcinomas were graded using the modified Bloom–Richardson classification. In addition to tumor type and grade, slides were examined for the presence of lymphovascular invasion, presence of ductal carcinoma in situ, and the status of axillary lymph nodes. Axillary lymph node status was determined on sentinel lymph node biopsy or axillary lymph node dissection. Status of estrogen receptor (ER) (6F11, Ventana Medical Systems, Oro Valley, AZ), progesterone receptor (PR) (IE2, Ventana Medical Systems, Oro Valley, AZ), and human epidermal growth factor receptor 2 (HER2) (4B5, Ventana Medical Systems, Oro Valley, AZ) was obtained from pathology reports for invasive cancers. Tumors were considered ER and PR positive when nuclear staining was present in greater than 1% tumor cells. HER2 status was determined using the 2013 American Society of Clinical Oncology/College of American Pathologists guidelines. Malignant lesions were classified into different molecular subtypes using immunohistochemical surrogates: Luminal A: ER and/or PR positive and HER2 negative; Luminal B: ER/PR positive and HER2 positive; HER2 positive: ER/PR negative, HER2 enriched; and triple negative/basal-like: ER/PR/HER2 negative (34).

Survival Outcomes

In all patients, date of progression (local recurrence, distant metastases) was recorded to determine the duration (months) of recurrence-free survival (RFS); and the date and cause of death or date of last follow-up was recorded to determine duration (months) of disease-specific survival (DSS). All patients underwent clinical and imaging follow-up (mammography, sonography and/or computed tomography) until progression, followed by routine follow-up until death. At the discretion of the treating physician, some patients were also followed with MRI of the breast and positron emission tomography/computed tomography scans. All local and distant recurrences were histopathologically verified.

Statistical Analysis

For statistical analysis, lesion characteristics were grouped into the categories as follows to determine the associations of MRS imaging biomarkers with tumor characteristics: benign versus malignant; tumor grade I, II, or III; nodal status positive or negative; and lymphovascular invasion present or absent. Additionally, molecular breast cancer subtypes were dichotomized into luminal A/B versus non-luminal (HER2 positive and triple negative) cancers and luminal A versus other (luminal B, HER2 positive and triple negative) cancers to determine associations with quantitative MRS imaging biomarkers. Categorical data were described using absolute numbers and percentages. For metric data, means (plus 95% confidence intervals) and standard deviations as well as median, minimum, and maximum were calculated. Due to skewed data, the latter were deemed to be more adequate. We performed the unpaired Mann–Whitney U-Test to compare lipid metabolites between groups. We further performed Bonferroni correction in groups that showed a statistically significant difference. In addition, we performed receiving operator characteristic (ROC) analyses; the best cut-off points were found using Youden’s index, and the sensitivity and specificity were computed based on the cut-off points. For survival analysis for disease progression, we performed the Kaplan–Meier test coupled with the log-rank test. All statistical tests were two-tailed and the significance was established at $p < 0.05$. The analysis for this study was generated using SAS v9.4 software (SAS Institute Inc., Cary, NC).

Results

Among 168 lesions, one hundred and forty-nine lesions enhancing lesions (size range, 8–87 mm; median, 21 mm) were identified. Of these, 89 were masses (size range, 8–70 mm; median, 20 mm) and 63 were non-mass enhancing lesions (size range, 8–87 mm; median, 27 mm).

Histopathology for all 168 patients revealed 111 lesions to be malignant (size range, 9–87 mm; median 21 mm) and 57 to be benign (size range, 8–60 mm; median, 20.5 mm). Among 111 malignant lesions, 97 lesions had ER and PR status, and 90 had HER2 status. Molecular breast cancer subtypes comprised 48 luminal A, 15 luminal B, 10 HER2 enriched, and 17 TN/basal-like lesions. Histopathologic diagnoses for all lesions are summarized in Table 1 and histopathologic details for malignant breast lesions are summarized in Table 2.

¹H-MRS for differentiation of malignant and benign breast tumors

The median and range of MRS voxel size among all breast lesions was 3.4 mL (range: 1–30 mL). The means values of various lipid metabolite concentrations were significantly lower in malignant lesions compared with that of benign lesions ($p=0.022$, 0.027 , and 0.0006 for L09, L21+L23, and L52+L53, respectively), while no significant difference was observed for L13+L16 ($p=0.0577$), L28 ($p=0.1196$), and L41+L43 ($p=0.1111$). After Bonferroni correction, only L52+L53 remained significant ($p=0.028$). None of the lipid metabolite concentrations were significantly different in malignant lesions stratified by histological grade, lymphovascular invasion, and axillary lymph node metastases (Table 3).

Figure 2 demonstrates the sensitivity of ¹H-MRS for quantifying lipid metabolism in breast lesions and figure 3 shows the ROC curves of all lipid peaks. The highest area under the curve (AUC) was derived for L52+L53 fat peaks (AUC=0.661), showing a sensitivity of 68.5% and a specificity of 63.2% (Table 4).

¹H-MRS in differentiation of molecular breast cancer subtypes

Differentiation of ER+/ER-, PR+/PR-, HER2+/-, lymphovascular invasion, and lymph node status—All lipid metabolite concentrations except for L41+L43 and L28 were significantly different between ER positive/ER negative and PR positive/PR negative malignant lesions. After Bonferroni correction, only L21+L23 remained significant in separating these groups ($p=0.046$). No significant correlation was found between any of the lipid metabolite concentrations and HER2 status, lymphovascular invasion, and metastasis status. (Table 3)

Luminal A vs other lesions—Among 90 malignant lesions with available hormone and HER2 receptor status, 48 (53.3%) were luminal A and 42 (46.6%) were other (luminal B/HER2 positive/triple negative) cancers. All lipid metabolite concentrations were significantly lower in luminal A cancers compared with other cancers (Table 3). After Bonferroni correction, only L21+L23 and L28 remained significantly different in separating these groups ($p=0.032$ for both). ROC curves are depicted in Figure 4, showing a best AUC value of 0.71 using L28, major lipid contributing to polyunsaturated fatty acid (PUFA) in

differentiating Luminal A from other lesions. Sensitivity and specificity values tabulated for all lipid peaks are shown in Table 4.

Luminal A/B vs non-luminal lesions—Among 90 malignant lesions with available hormone and HER2 receptor status, there were 63 (70%) luminal A/B cancers and 27 (30%) non-luminal (HER2 enriched/TN) cancers. All six lipid metabolite concentrations were significantly lower in luminal A/B cancers compared with non-luminal cancers (Table 3). After Bonferroni correction, only L28 remained significantly different between these cancer groups ($p=0.046$). ROC curves are shown in Figure 5, with the best AUC value of 0.77 using L28, major lipid contributing to PUFA in differentiating Luminal A/B from other lesions. Sensitivity and specificity values tabulated for all lipid peaks are shown in Table 4.

The feasibility of separating adjacent lipid resonances with a chemical shift difference less than 0.2 ppm was tested at higher field strengths such as 3T (example spectra is shown in Figure 6). It is clearly visible that L21 and L23 can be accurately separated due to increased resolution at 3.0T compared with 1.5T, facilitating accurate determination of unsaturated and saturated fatty acid fractions. STEAM sequence with short echo time also resulted in higher lipid signal sensitivity.

Survival outcomes—The mean follow-up for all patients was 52.5 months (range 0 and 172 months). Fifty-five patients (32%, 55/168, 20 benign and 35 malignant) did not undergo the annual clinical and/or imaging at our institution and were therefore considered lost to follow-up.

Among breast cancer patients, eighteen (16%, 18/111) progressed during the follow-up period: 4/18 (22%) local, 2/18 (11%) regional, and 12/18 (66%) distant recurrence. Mean recurrence free-survival, expressed as months from surgery to recurrent disease, was 80.5 (range, 0–190) months.

Seven (4%, 7/168) patients died during the follow-up period and 3 out of the 7 patients died of breast cancer during the follow-up period at a median interval of 59 months (range, 10–157 months).

Patients who presented with disease recurrence and/or succumbed to breast cancer had significantly lower lesion L09, L13+L16, L21+L23 ($p<0.0001$) and L28 ($p=0.0027$) lipid metabolites compared with patients without recurrence on long-term follow-up. Patients were split into two groups based on median values for the 6 peaks. Kaplan–Meier analysis showed that there were significant differences in recurrence-free survival between the two groups for L09 ($p=0.0173$), L21+L23 ($p=0.0024$), and L28 ($p=0.0045$) where p-values were computed using log-rank test. No significant differences in L13+L16 ($p=0.0501$), L41+L43 ($p=0.2051$) and L52+L53 ($p=0.8366$) were observed in recurrence-free survival between two groups (Figure 7).

Discussion

The reprogramming of cellular metabolism is a recognized cancer hallmark (35,36). There is an increasing interest in lipid metabolism alterations during cancer development as potential

targets for breast cancer diagnostics and therapeutics. In this *in vivo* breast MRS study, we investigated whether lipid metabolite concentrations detected with ^1H -MRS can be used for the non-invasive differentiation of benign and malignant breast tumors, for differentiation among molecular breast cancer subtypes, and for prediction of long-term survival outcomes. We show that breast cancers have significantly lower unsaturated fatty acid lipid L52+L53 concentrations compared with benign breast tumors. Our data suggest that based on lipid metabolites, malignant tumors can be differentiated from benign lesions with good sensitivity and specificity. Lipid metabolites L09 and L21+L23 were also significantly lower in malignant versus benign tumors.

Our findings are in good agreement with the preliminary results by He et al. (37). They used ^1H -MRS at 2.1T with selective multiple quantum editing techniques, demonstrating reduced olefinic methylene protons ($-\text{CH}=\text{CH}-$) at 5.3 ppm (L53) of poly-unsaturated fatty acid coupled with allylic methylene protons ($-\text{CH}_2-\text{CH}_2-\text{CH}-$) of unsaturated acyl chain at 2.8 ppm (L28) in patients with invasive ductal carcinoma compared with patients with normal breast parenchyma. However, these sequences do not allow the assessment of all lipid peaks within a single proton MRS scan and are not readily available on clinical MR scanners.

To date, the application of MRS in breast imaging for an improved diagnosis and characterization of breast cancer has been largely based on the detection and spatio-longitudinal monitoring of Cho (9–20,38) and the full potential of MRS in this context has not been explored. Initial studies investigated whether water and fat (L13+L16) metabolites in addition to choline was useful for breast cancer diagnosis. These yielded promising results. More recent studies reported that the L13+L16 peak (21) or fat fraction (26) is significantly smaller in malignant lesions than in normal breast tissue. While these studies observed no significant difference in water/fat ratios in benign lesions and normal parenchyma, the AUC of the water/fat ratio for was significantly higher in malignant lesions, reflecting lower L13 peak area and increased tumor tissue water levels. This can be explained by a reduction in the volume of the fat fraction in breast cancer caused by obliteration/replacement of fatty tissue during tumor growth, with similar results having been reported mouse models (39).

The current study expands on this topic by investigating additional different fatty acid peaks such as L21+L23, L28, L41+L43, and L52+L53 resonances in addition to L13+L16. We demonstrated that mean values of L09, L21+L23, L52+L53 lipid concentrations were significantly lower in malignant compared with benign lesions while no significant difference was observed for L28, L41+L43, and L13+L16 lipid concentrations. Our data indicated that the quantitative *in vivo* ^1H -MRS L52+L53 MRS parameter is the most promising biomarker, yielding the highest AUC for differentiating malignant and benign tumors and remaining significant after Bonferroni correction.

In our study, we also investigated the potential of quantitative *in vivo* ^1H -MRS for molecular subtype differentiation, i.e., luminal A/B cancers versus other cancers and luminal A versus other cancers. The differentiation between luminal cancers and other cancers is of paramount clinical importance since luminal cancers are treated with endocrine therapy and benefit less from cytotoxic chemotherapy. Therefore, quantitative *in vivo* ^1H -MRS

assessment of lipid metabolism as a non-invasive imaging biomarker may add relevant information to help guide therapeutic decisions. Mean values of all lipid peaks were significantly different, with ROC analysis yielding the highest AUC for the L28 lipid peak. Results on poly unsaturated lipid peaks have been shown to be useful (37), which is in good agreement with our results.

In addition, we investigated the associations of quantitative *in vivo* ¹H-MRS assessment of lipid metabolism with RFS and DSS. On long-term follow-up with a median of 52.5 months, patients with higher lipid metabolite concentrations showed higher progression-free survival rates, in particular, with statistical significance for L09, L21+L23, and L28, indicating that tumor lipid metabolite concentrations may serve as an imaging biomarkers of tumor aggressiveness. The results of the current study further highlight that functional MRI features can improve our understanding and prediction of cancer progression.

The findings of our study support the use of quantitative *in vivo* ¹H-MRS for assessment of lipid metabolism. This may provide an additional imaging biomarker for breast cancer diagnosis, characterization and prognosis with the ultimate goal of implementation in the clinical routine. At higher field strengths, i.e., 3T and 7T, an improved assessment of L21, L23, L41, and L43 peaks with higher signal sensitivity and more accurate calculation of saturate and unsaturated fatty acid fractions would be facilitated (26,40); therefore, prospective studies at higher field strengths are warranted to validate these results. In addition, these metabolites relate to obesity that has been linked to cancer development (41). Therefore, quantitative *in vivo* ¹H-MRS assessment of lipid metabolism may in this context help to elucidate the underlying oncogenic processes. However, challenges for its implementation in clinical practice have to be addressed such as the need for standardization and the establishment of normal baseline values of lipid metabolites at higher fields such as 3T that are increasingly used in clinical practice. The concentrations of lipids can vary in different regions of the healthy breast (fatty vs glandular) and has been widely discussed in the MRS literature (42). However, the goal of this present study is not affected by this constraint as we focused on fat composition of lesion itself.

There are limitations to our study. Firstly, this was a retrospective analysis from a prospective database which limited the establishment of a threshold to define the accuracy of to differentiate malignant lesions using lipid peaks. Secondly, this study was performed at 1.5T to assess choline, an established biomarker for breast malignancy. 1.5T MRS signals used for choline detection with a long echo time (TE=135 ms) may affect the lipid metabolite signals due to T2 relaxation losses (28); however, using various lipid MRS peaks, we observed a positive trend in identifying breast cancer. In the future, our goal is to quantify MRS-visible lipid (43) metabolites prospectively. In our study, we have used our retrospective data collected at a TE of 135ms originally optimized for choline detection without taking into account relaxation effects to provide an initial understanding of different MR-visible lipid level changes in malignant lesions compared with benign lesions and among different types of molecular subtypes. It can be expected that further prospective studies with a different acquisition sequence such as STimulated Echo Acquisition Mode with a short echo time will confirm our initial findings.

In conclusion, quantitative *in vivo* ^1H -MRS assessment of lipid metabolism enables the differentiation of benign and malignant breast tumors, differentiation among molecular breast cancer subtypes and prediction of long-term survival outcomes and thus may provide an additional non-invasive imaging biomarker to guide therapeutic decisions in breast cancer.

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Fig. 2. Sensitivity of proton MRS for lipid metabolism in breast lesions. Left Panel: Representative magnetic resonance (MR) image and (Point RESolved Spectroscopy) PRESS spectra from a mammographically detected biopsy proven invasive ductal carcinoma from a 52-year-old woman. **A.)** Sagittal, T1-weighted, MRI of the left breast immediately after injection of intravenous gadolinium with magnetic resonance spectroscopy (MRS) voxel covering the lesion. **B.)** *In-vivo* experimental MR spectra from the tumor voxel (blue) along with the LC-model fit (red), residuals (green), and individual lipid peaks (black). There is a visible positive choline peak. **Right Panel:** 43-year-old female presented with a new palpable mass in the right breast. MR-guided biopsy followed by surgical excision yielded benign fibrosis and ductal hyperplasia. **C.)** Post contrast sagittal T1-weighted MRI. **D.)** *In-vivo* experimental MR spectra from the tumor voxel (blue) along with the LC-model fit (red), residuals (green), and individual lipid peaks (black). There is no visible positive choline peak

Fig. 3. Differentiation accuracy of malignant breast lesions.

Receiver operating curve (ROC) of lipid metabolites showing the highest area under the curve (AUC) for the L52+L53 lipid peak

Fig. 4. Differentiation accuracy of molecular subtypes (luminal A versus other cancers).
ROC Receiver operating curve (ROC) of all six fat metabolites

Fig. 5. Differentiation accuracy of molecular subtypes (luminal A/B versus other cancers).
Receiver operating curve (ROC) of lipid metabolites

Fig. 6. Lipid magnetic resonance spectroscopy (MRS) at 3.0 Tesla field strength demonstrating peaks with increased separation and sensitivity.

Representative magnetic resonance (MR) image and STimulated Echo Acquisition Mode (STEAM) spectra from a control subject. **A.)** Reformatted sagittal, T1-weighted, pre-fat saturated MRI of left breast with MRS voxel covering the fibroglandular tissue (FGT). **B.)** In-vivo experimental MR spectra from the FGT voxel (blue) along with the LC-model fit (red), residuals (green), and individual lipid peaks (black). Clear identification of L21 and L23 peaks can help in calculating unsaturated fatty acid fractions in breast tissue which is found in obesity-related diseases.

Fig. 7. Kaplan–Meier curves for recurrence-free survival.

Patients were split into two groups based on the median value for each lipid metabolite as indicated.

Table 1.

Characteristics of breast lesions

Characteristics of lesions	N	%
<i>Malignant lesions</i>		
Number of lesions	111	100.0
Mean size (range), cm	2.1 (0.9-8.7)	
<i>Lesion type</i>		0.0
Mass	79	71.2
Non-mass	30	27.0
Not available	2	1.8
<i>Histopathological type</i>		
Invasive ductal carcinoma	83	74.8
Invasive lobular carcinoma	10	9.0
Ductal carcinoma <i>in situ</i>	12	10.8
Other	6	5.4
<i>Benign lesion</i>		
Number of lesions	57	100.0
Mean size (range), cm	2.1 (0.8-6.0)	
<i>Lesion type</i>		0.0
Mass	10	17.5
Non-mass	30	52.6
Not available	17	29.8
<i>Histopathological type</i>		
High risk lesions	9	15.8
Lobular carcinoma <i>in situ</i>	5	8.8
Atypical lobular hyperplasia	1	1.8
Atypical ductal hyperplasia	3	5.3
Benign lesions	48	84.2
Breast parenchyma	20	35.1
Fibroadenoma	6	10.5
Fat necrosis	1	1.8
FCC	5	8.8
FCC, SF, inflammation	1	1.8
FCC, SF, PASH	1	1.8
PASH	2	3.5
PASH, DH, CCC, apocrine metaplasia	1	1.8
SA, SF, FCC	10	17.5

CCC, columnar cell changes; DH, ductal hyperplasia; FCC, fibrocystic changes; PASH, pseudoangiomatous stroma hyperplasia; SA, sclerosing adenosis; SF, stromal fibrosis

Table 2.

Histologic characteristics of malignant breast lesions

Characteristics	n	%
<i>Total malignant lesions</i>	111	100
Lymph node metastases		
Positive	41	37.5
Negative	51	46.4
n/a	19	16.1
Lymphovascular Invasion		
Present	36	33
Absent	46	42
n/a	29	25
Histological grade		
I	2	1.8
II	21	19.6
III	59	53.6
n/a	29	25
ER		
Positive	68	60.7
Negative	29	26.8
n/a	14	12.5
PR		
Positive	57	35.7
Negative	40	51.8
n/a	14	12.5
HER2		
Positive	64	58.9
Negative	26	22.3
n/a	21	18.8
Subtypes		
Luminal A	48	42.9
Luminal B	15	13.4
HER enriched	10	8.9
Triple negative/basal-like	17	15.2
n/a	21	19.6

ER, estrogen receptor; HER2, human epidermal growth factor receptor

Table 3.

Mann–Whitney U tests for lipid metabolites between different groups

p-value	ER+ versus ER– (n=68/29)	PR+ versus PR– (n=57/40)	HER2+ versus HER2– (n=64/26)	LVI+ versus LVI– (n=36/46)	LN+ versus LN– (n=41/51)	Malignant versus Benign (n=111/57)	Luminal A/B versus Others (n=63/27)	Luminal A versus Others (n=48/42)
L09	0.002	0.002	0.702	0.358	0.357	0.022	0.0088	0.0057
L13+L16	0.002	0.003	0.572	0.298	0.297	0.058	0.0044	0.0020
L21+L23	0.001	0.001	0.572	0.363	0.684	0.027	0.0012	0.0007
L28	*	*	0.352	0.645	0.414	0.119	0.0001	0.0007
L41+L43	0.062	0.077	0.510	0.285	0.784	0.201	0.0273	0.0132
L52+L53	0.021	0.074	0.319	0.46	0.701	0.001	0.0121	0.0022

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor; LN, lymph node; LVI, lympho vascular invasion; n, number of lesions in each group.

* Not enough patients

Table 4.

Sensitivity and specificity values calculated from ROC curves

<u>Malignant versus Benign</u>			<u>Luminal A versus other cancers</u>		<u>Luminal A/B versus other cancers</u>	
Variable	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity
L09	0.75(0.54–0.88)	0.47(0.27–0.59)	0.55(0.23–0.70)	0.79(0.50–0.90)	0.65(0.30–0.83)	0.69(0.35–0.85)
L13+L16	0.77(0.56–0.88)	0.42(0.19–0.53)	0.55(0.17–0.68)	0.81(0.52–0.93)	0.91(0.60–0.97)	0.42(0.08–0.58)
L21+L23	0.70(0.51–0.82)	0.50(0.32–0.60)	0.55(0.19–0.72)	0.86(0.60–0.95)	0.67(0.41–0.81)	0.69(0.35–0.85)
L28	0.84(0.60–0.93)	0.34(0.12–0.44)	0.60(0.36–0.74)	0.79(0.43–0.93)	0.83(0.56–0.90)	0.65(0.26–0.85)
L41+L43	0.54(0.30–0.68)	0.64(0.42–0.73)	0.66(0.36–0.79)	0.64(0.26–0.76)	0.65(0.37–0.83)	0.62(0.35–0.81)
L52+L53	0.63(0.28–0.74)	0.69(0.45–0.76)	0.77(0.53–0.89)	0.57(0.24–0.71)	0.62(0.22–0.81)	0.77(0.50–0.92)

95% confidence intervals are specified within the parentheses next to the actual sensitivity or specificity