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CEST-Dixon for human breast lesion characterization at 3T: a preliminary study

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Abstract

Purpose—CEST MRI for breast lesion characterization is promising. However, artifacts are prone to develop in breast CEST imaging due to strong lipid signals. The aims of the study are: 1) to develop and validate the CEST-Dixon imaging sequence for simultaneous water-fat separation and B_0 mapping; 2) use the CEST-Dixon method to characterize suspicious lesions in patients undergoing percutaneous biopsy.

Methods—The gradient-echo multi-echo Dixon acquisition is used to create fat-free CEST and B_0 maps. The sequence has been validated in phantoms and in vivo. Five healthy volunteers and ten patients were scanned to compare the CEST contrast in three frequency ranges centered at 1, 2 and 3.5 ppm. The correlation between the CEST contrast and pathology markers (tumor type, ER status, Ki-67) was also investigated by stratifying the patients into ER-negative invasive ductal carcinoma (IDC) (more aggressive), ER-positive IDC (less aggressive) and benign groups.

Results—The CEST-Dixon sequence shows homogenous fat removal in the water-only images. The ER-negative IDC tissues display a trend to higher CEST contrast in all three frequencies ranges, while the ER-positive IDC, benign and normal tissues have lower CEST contrast. No significant differences were observed between the ER-positive IDC, benign and normal tissues. Of the three frequencies ranges, the CEST contrasts at 1 ppm are high in the ER-negative IDC group, have the largest difference between the ER-negative IDC and the other groups and have the highest correlation with Ki-67.

Conclusions—Breast CEST-Dixon imaging shows potential to differentiate more aggressive from less aggressive cancers.

Keywords

CEST; Dixon; breast cancer; breast MRI; estrogen receptor; Ki-67

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Introduction

Magnetic Resonance Imaging (MRI) has been adopted as both a screening tool for women at high risk for developing breast cancer as well as a diagnostic tool to evaluate the extent of disease in women recently diagnosed with breast cancer (1,2). Dynamic contrast enhanced (DCE) breast MRI provides very high sensitivity but lacks specificity (3) due to significant overlap in the enhancement patterns of benign and malignant breast lesions (4). Multiple non-contrast imaging methods, such as spectroscopy and diffusion-weighted imaging, have been developed and applied to breast MRI in order to improve specificity and decrease false positive results when used either alone, or in combination with DCE-MRI (5).

Recently, chemical exchange saturation transfer (CEST) has been investigated for its feasibility and application in cancer imaging, because it provides information at the molecular level that reflects biochemical composition of tissues (6). Compared with spectroscopy, CEST has significantly improved sensitivity to certain metabolites and can achieve much higher spatial resolution, which is particularly desirable for clinical applications (6). Amide Proton Transfer (APT) neuroimaging, a sub-type of CEST imaging, is emerging as a powerful tool in the assessment of brain tumor aggressiveness and treatment response monitoring (7,8). Several preliminary studies in cells, animals, and humans applied CEST to breast malignancies and also demonstrated the potential for tumor detection, characterization, and treatment assessment (9–14). Therefore, addition of CEST to current breast imaging protocols may potentially lead to technology with improved specificity and prediction value, while retaining excellent sensitivity.

Most of the reported studies on breast CEST imaging are preclinical or focus on imaging sequence implementation and/or optimization (9,12–14). Only two papers on CEST imaging have incorporated small number of breast cancer patients (10,11). Many questions need to be further investigated with regards to the application of CEST to breast malignancy, including how CEST contrast changes in breast tumors, which CEST frequency range has the highest correlation with malignancy, and what are the major contributors to CEST contrast in malignancy.

One of the largest challenges for successful breast CEST imaging is the presence of a large fat signal. Although the total amount of fat varies in the breast, fibroglandular tissue and fat are interleaved. Fat can confound CEST contrast, complicate Z-spectrum appearance and potentially lead to lipid artifacts and erroneous CEST effects (15,16).

In this study, we discuss a CEST-Dixon method that employs the combination of CEST preparation with multi-point Dixon water-fat separation to obtain water-only CEST images (17,18). These water-only images are used to calculate MTR_{asym} maps in three CEST frequency ranges for normal tissues and suspicious lesions. Biopsy results were used as the gold standard for method evaluation. The goal of the study includes three aspects: 1) to implement and validate the CEST-Dixon sequence in human subjects at 3T for simultaneous water-fat separation and B_0 mapping, 2) to compare the CEST contrasts in normal breast tissues with that of malignant and benign tissues in three CEST frequency ranges, and 3) to

determine if the CEST-Dixon method could be used to assess tumor status using several standard biopsy pathology markers (ER and Ki-67 (19)) as reference standards.

Methods

Phantom

To investigate whether CEST-Dixon introduces additional asymmetries in the Z-spectrum, iopamidol (Isovue-300, Bracco Diagnostics, Milan, Italy) phantoms at pH 6.0, 6.5, 7.0 and 7.5 were prepared and transferred to small vials. A vial containing vegetable oil (Crisco, the J.M. Smucker Co, Orrville, OH) was used as a fat reference. A vial of distilled water was prepared as a control. The vials were held in a plastic container that was filled with tap water to minimize B_0 inhomogeneity.

Subjects

The study was approved by the local Institutional Review Board (IRB) and performed in accordance with the guidelines. Written consent was obtained from each subject. 5 female volunteers without known breast diseases and 10 female patients with suspicious breast lesions for which biopsy was recommended were recruited for the study. The patients were scanned prior to biopsy and the diagnostic “gold standard” was provided via clinical pathology results, which included 8 malignancies and 2 non-malignant lesions. The confirmed malignancies included 6 invasive ductal carcinoma (IDC), 1 IDC and encapsulated papillary carcinoma (EPC), and 1 invasive mucinous carcinoma (IMC) (Table 1). The non-malignant lesions included 1 atypical ductal hyperplasia (ADH) and 1 fibroadenoma (Table 1). Only one lesion was scanned per patient. Patient 7 was excluded from the analysis due to observable motion since the tumor was located close to the chest wall. Patient 8 was excluded from the analysis due to the small size of the lesion (which at the acquired image resolution ($2 \times 2 \text{ mm}^2$) resulted in partial volume effects and only few pixels for the ROI analysis). Patient 2 was excluded from the analysis because IMC and IDC are two different tumor types. Thus, the analysis included 5 healthy volunteers and 7 patients with suspicious breast lesions in total. The patients are further stratified into three groups: ER-negative (ER-) IDC group (Patient 1 and 3, $N = 2$), ER-positive (ER+) IDC group (Patient 4–6, $N = 3$) and benign group (Patient 9 and 10). The ER- breast cancer lack the estrogen receptor which is an index for sensitivity to endocrine treatment and is more aggressive than the ER+ breast cancer (20).

MRI

A 3T human scanner (Ingenia, Philips Healthcare, Amsterdam, the Netherlands) with dual-channel body coil was used throughout the study. The phantom data were acquired using a 15-channel head-spine coil. The CEST preparation consisted of 10 hyperbolic secant (HS) pulses, each 49.5 ms long, flip angle (FA) of 900° and with a delay of 0.5 ms between the pulses. The total saturation time was 500 ms and $B_{1,CW} = 1.2 \mu\text{T}$. The CEST images were acquired using 2D 3-point multi-echo Dixon sequence with $TR/TE_1/TE_2 = 4.8/1.33/1.1 \text{ ms}$, $FA = 45^\circ$ and centric k-space ordering. The Dixon acquisition was based on a multi-shot multi-echo T_1 -weighted turbo field echo (TFE) sequence. We chose 3 echoes (3-point Dixon method, Fig. 1), since it is the minimal number of the echoes required to obtain water, fat

and B_0 information. 41 offsets were acquired in the Z-spectrum from -10 ppm to 10 ppm and 1 reference image was acquired. The FOV was 220×220 mm² with a voxel size of 1.5×1.5 mm² and a slice thickness of 8 mm.

The human data were acquired using a 16-channel bilateral breast coil. A 3D fat-suppressed enhanced T_1 high-resolution isotropic volume excitation (eTHRIVE) sequence with a resolution of $0.6 \times 0.6 \times 1$ mm³ was used for anatomical images. The imaging slice was placed for optimal observation of the fibroglandular tissue and/or the suspicious lesion based on the eTHRIVE images. The CEST preparation and acquisition was the same as the phantom experiment except for the following parameters: TR/TE₁/TE = 5.1/1.57/1.1 ms, FA = 10° and 33 offsets were acquired in the Z-spectrum from -6 ppm to 6 ppm. The FOV was varied according to the volunteer size, but the in-plane resolution was fixed at 2×2 mm² and the slice thickness was 5 mm. Transvers bilateral images were acquired. The data from the first two patients were acquired with SENSE = 4 and multi-shot factor 14 and the total scan time 1 min 10 s. All the other volunteer and patient data were acquired without SENSE and using multi-shot factor 25 to improve SNR, and the total scan time was 2 min 26 s.

Data analysis

6 types of images were obtained using the Dixon method for each frequency offset: 1) the source images (three images total, one per TE), 2) the water-only image, 3) fat-only image, 4) in-phase image (IP), 5) out-of-phase (OP) image, and 6) the B_0 map. Standard single-peak lipid Philips reconstruction protocol was used to obtain water-only, fat-only, IP, OP and B_0 images from the source images.

Phantom studies—To investigate whether CEST-Dixon introduces additional asymmetries in the Z-spectrum, the MTR_{asym} at 1.8, 4.2 and 5.5 ppm (21) calculated from the CEST images acquired with and without Dixon were compared in the phantom study.

In vivo studies—The water-fat separation of the CEST-Dixon method was validated in vivo by comparing the Z-spectra from the CEST images acquired with and without Dixon for pixels of different fat fractions. The second echo source images (TE = 2.43 ms and 2.67 ms for phantom and in vivo studies respectively, both are close to IP) were chosen to serve as a standard, non-Dixon CEST images for the comparison. The Dixon CEST images were the water-only images.

The water-only CEST images were processed on a pixel-by-pixel basis using custom Matlab (The Mathworks, Natick, MA) routines. Field inhomogeneity was corrected using an averaged B_0 map generated by averaging the Dixon B_0 maps of all frequency offsets. MTR_{asym} was used for CEST signal measurement. For in vivo studies, the MTR_{asym} were averaged in the ROIs and in the three frequency ranges: 1) 0.8–1.2 ppm, 2) 1.8–2.2 ppm, and 3) 3.3–3.7 ppm and denoted hydroxyl, amine and amide MTR_{asym} respectively.

ROI placement: Healthy volunteers—ROIs of the fibroglandular tissue of the healthy volunteers were made based on the signal intensities of the water-only and fat-only images (10). First, a threshold was selected to exclude the background noise in both water-only and fat-only images. The two masks were then overlapped to locate the pixels affected by partial

volume effects and with high fat fraction (FF). The fat fraction was calculated pixelwise based on the water-only and fat-only images: $FF = W/(W+F)$. The high FF pixels were removed from the mask based on the water-only image. Next, skin, chest wall and heart were removed from the mask manually to generate the final mask for the fibroglandular tissues.

Patient volunteers—The tumor ROIs were drawn manually by a fellowship-trained breast imaging radiologist with 5 years of experience based on the water-only images while referring to the high resolution eTHRIVE images. The final tumor and fibroglandular tissue ROIs were reviewed and approved by the radiologist.

Statistical analysis

A weighted Least Squares Linear fit was performed for the correlation between MTR_{asym} and Ki-67 level with the inverse of the square of the standard deviation used as the weighting factor (22), and a $p < 0.05$ was considered statistically significant.

Results

Figure 2 compares the MTR_{asym} at 1.8, 4.2 and 5.5 ppm of the water and iopamidol phantoms calculated from the images acquired with and without Dixon. The non-Dixon and Dixon images refer to the second echo source image and water-only images. As shown in Figure 2, the MTR_{asym} with and without Dixon are similar for all the phantoms and frequencies, although Dixon results showed slightly increased effect in the iopamidol solutions (the values are still within the experimental error of each other).

To validate the water fat separation of CEST-Dixon method, Figure 3 shows the three types of images obtained from a healthy volunteer: the second echo source image, water-only and fat-only images. Water-only and fat-only images are of a good quality, and provide a clear separation of water and fat signals (Fig. 3n,o vs m). Figure 3 also shows the in vivo Z-spectra for single pixels with different fat fractions in the 3 types of images. The selected single pixels are shown as colored dots in Figure 3h inset. The pixels were selected from a straight line going from fibroglandular tissue to the fat with an increasing fat fraction along the line. Hence, the water only pixel was from the fibroglandular tissue, the fat only pixel was from the fat tissue and the pixels with mixed water fat signals were selected at the interface of the two tissues. Since the second echo source images ($TE = 2.67$ ms) were close to IP images, the water dip in the Z-spectra decreases while the fat dip increases as the fat fraction increases (Fig. 3a–d) (15). The corresponding water-only and fat-only Z-spectra are shown in the Figure 3e–h and Figure 3i–l respectively. It can be seen that the water and fat dips were successfully separated, though some residual fat signals were observed in high fat fraction (Fig. 3g arrow). The Z-spectra were not normalized to the reference in this figure to better display the pixels whose signal intensities were close to noise level: fat-only pixel in the water-only images (Fig. 3h) and the water-only pixel in the fat-only images (Fig. 3i).

Figure 4 shows the amide CEST maps with and without Dixon and the corresponding whole fibroglandular tissue ROI averaged Z-spectra and MTR_{asym} of the same healthy volunteer as Figure 3. Here, the ROI encompassing the whole fibroglandular tissue was generated as

described above (“Methods, ROI placement, healthy volunteers”) and is shown in Figure 4g. The non-Dixon images refer to the second and first echo source images, which are close to the in-phase (IP, TE = 2.67 ms) and out-of-phase (OP, TE = 1.57 ms) images, respectively. Dixon results shown are the water-only images. As shown in Figure 4a, in non-Dixon image, a large number of pixels in the amide CEST map have negative values due to the presence of fat (15). Correspondingly, a fat dip is observed in the Z-spectrum, leading to negative amide MTR_{asym} (Fig. 4b). Non-Dixon images acquired close to OP condition lead to curious looking Z-spectrum and erroneously high MTR_{asym} (Fig. 4d,c) due to signal interferences and normalization that was discussed in an earlier publication (15). At the same time, in the water-only Z-spectrum (Fig. 4e), the fat dip is removed, producing the amide MTR_{asym} and CEST map (Fig. 4e,f) essentially free of fat influence. To demonstrate the degree of B_0 inhomogeneity that is obtained in a typical case, Figure 4h shows the average B_0 map obtained in the same healthy volunteer. Large deviations in the homogeneity spanning a range from +200 to -200 Hz can be observed.

Figure 5 shows the representative water-only Dixon hydroxyl CEST maps and ROI averaged Z-spectra and MTR_{asym} for a healthy volunteer (Fig. 5a,b), an IDC, Not Otherwise Specified (NOS), ER+ patient (Fig. 5c,d) and a triple-negative breast cancer (TNBC) patient (Fig. 5e,f). The Z-spectrum and MTR_{asym} of the healthy volunteer was averaged across the fibroglandular tissue of both breasts (Fig. 5b). The Z-spectra and MTR_{asym} of the patients were averaged in the tumor areas indicated by the white arrows (Fig. 5c,e). The MTR_{asym} in the three CEST frequency ranges of all the subjects are summarized in Figure 6 and Supporting Figure S1. When the malignant lesions are stratified by ER status, it can be seen that the ER- IDC group exhibits higher CEST effects in all the three frequency ranges than the ER+ IDC, benign and normal groups (Fig. 6). For the three frequency ranges, the ER+ IDC, benign and normal groups tend to have similar CEST effects which suggest that the ER + IDC group is indistinguishable from normal and benign groups. Moreover, the hydroxyl range has the largest difference between the ER- IDC and the other groups. Hence, Figure 5 displays hydroxyl MTR_{asym} . When NOT stratified by ER status, although the IDC group displays higher MTR_{asym} in hydroxyl and amine ranges than the other groups, the deviations across subjects increase (Supporting Fig. S1).

In Figure 7, the tumor MTR_{asym} in the three frequency ranges are plotted against the Ki-67. It can be seen that the MTR_{asym} increases as Ki-67 level increases for all the frequency ranges. The R^2 values were 0.95, 0.87 and 0.36 for hydroxyl, amine and amide frequency ranges, respectively.

Figure 8 displays the MTR_{asym} in the three frequency ranges for all subjects, similar to Figure 6, but using the second echo source images. The hydroxyl and amine ranges still have the highest values in the ER- IDC group, however the amine MTR_{asym} had decreased in ER + IDC, benign and normal groups (Fig. 8 vs Fig. 6). This is due to the influence of the lipid signals on the amine frequencies. Moreover, the APT signal becomes negative for normal, benign and ER+ lesions, due to increased negative lipid contribution.

Discussion

The presence of strong lipid signals is a big challenge for body CEST imaging because fat can lead to erroneous CEST contrast. CEST-Dixon offers attractive approach around this obstacle. The B_0 maps derived from the Dixon technique can be used for field inhomogeneity correction, without the need for a separate B_0 mapping sequence. In this study, a 3-point multi-echo Dixon is used for image acquisition. 3 TE values were chosen because this is the least number of echoes needed to robustly separate water, fat, and B_0 .

TE was adjusted to the minimum possible value to reduce potential artifacts caused by phase-wrapping.

The CEST-Dixon method was validated in phantoms and in vivo. In the phantom experiment, the MTR_{asym} with and without Dixon are similar (Fig. 2) indicating that the Dixon water fat decomposition does not introduce additional asymmetries to the Z-spectrum. The vial containing fat only was not visible in the water-only images. Moreover, Figure 2 indicates that MTR_{asym} measurements using Dixon images lead to slightly reduced standard deviation. This might be due to the multi-point Dixon post-processing involving 3 echoes acting similar to averaging, thus increasing SNR.

The in vivo results of the healthy volunteers show successful water-fat separation (Fig. 3m–o). However, in some of the pixels with high fat fraction (approximately > 50%), the lipid peak was still detectable in the water-only images (Fig. 3g, arrow), albeit much smaller than what is observed in the non-Dixon image (compare Figs. 3c vs 3g). This residual fat contribution in the Z-spectrum would be magnified by normalization (15), hence, MTR_{asym} distortions might still be observed. For this reason, in this preliminary study, the pixels with high fat fraction were removed from the ROIs to avoid the influence of the residual fat. While we cannot conclusively indicate the origin of this residual artifact, partial volume effects might be a contributing factor (10). Another factor should also be considered: the fat-water separation model of multi-point Dixon post-processing assumes non-saturated water and fat peaks, which is not true in CEST. Also, since the single-peak model for fat was used in the image reconstruction, other fat peaks would remain in the Z-spectrum and introduce additional asymmetries, especially the second and third largest fat peaks which lie about 3.8 ppm upfield and 0.6 ppm downfield respectively may influence the amide and hydroxyl MTR_{asym} accordingly. Additional investigation is needed on the influence of the saturation and multi-peak vs single-peak modeling on CEST-Dixon method. Finally, it might be a contribution from the true relayed Nuclear Overhauser Effect (NOE), cleared from the artifact contribution of the saturated, but non-exchanging lipids.

Figure 4e,f demonstrates that despite some residual problems described above, CEST-Dixon leads to a smoother Z-spectrum in the normal fibroglandular tissue and while residual lipid artifacts might be observable in some pixels, overall their influence is removed. Moreover, the Z-spectrum from water-only images displays higher suppression levels near water resonance, almost zero, as should be expected (Fig. 4f). While second echo (close to IP) leads to higher values close to water resonance, due to fat signal contribution (Fig. 4a).

Following validation of the CEST-Dixon method in the healthy volunteers, a preliminary study was conducted in the small group of patients with suspicious breast lesions identified at mammography and ultrasound. Figures 5–6 and Supporting Figure S1 demonstrate that the MTR_{asym} in the healthy fibroglandular tissue is generally low, around 2% for all three frequency ranges. Comparing to the previously reported human breast studies at 3T, the amide MTR_{asym} is slightly lower than the previously reported values (10,14), however MTR_{asym} at 1.2, 1.3 and 1.8 ppm reported earlier was mostly negative (11), presumably due to lipid contribution. The standard deviations within ROI that included whole fibroglandular tissue (with high fat fraction pixels excluded), were around 4% (with minimum 2% and maximum 5% observed). This relatively large standard deviation is in line with previous CEST measurements in breast at 3T and at 7T (10,13,14).

Table 1 lists MTR_{asym} values measured in the patients. The standard deviations with ROIs are similar to the ones observed in healthy volunteers. The data was stratified by ER status (ER– vs ER+). Figure 6 suggests that such stratification provides differentiation between the more aggressive and less aggressive cancer groups. In ER– IDC tissues, there is a trend towards increased MTR_{asym} compared to the ER+ IDC, benign and normal tissues especially in the hydroxyl and amine ranges (Fig. 6). The ER+ IDC and benign lesions demonstrate MTR_{asym} values close to normal fibroglandular tissue in all three frequency ranges. When both ER statuses are grouped together, the trend of increased MTR_{asym} in the IDC group, as compared to the other groups, can still be seen in the hydroxyl and amine ranges, however this trend is reduced (Supporting Fig. S1). In the amide range, the IDC group becomes indistinguishable from the benign and normal groups (Supporting Fig. S1). Although the sample sizes are very small, this preliminary data re-iterates important potential for CEST MRI to differentiate more aggressive from less aggressive breast cancers.

A statistically significant correlation was observed between Ki-67 and MTR_{asym} in all the three frequency ranges with the most significant correlation for hydroxyl MTR_{asym} and amine MTR_{asym} close behind (Fig. 7). Our study is small and the Ki-67 values reported here do cluster at the low and high end. Nevertheless, to the best of our knowledge, correlation of Ki-67 and APT was observed previously in animal model of brain cancer (23), but our results are the first to demonstrate such correlation in humans. High Ki-67 indicates increased cell proliferation. While it is not an accepted marker of breast cancer aggressiveness, it is one of the standard pathological indices evaluated for patient care and is associated with more aggressive cancer types (24). The observed correlation is in agreement with our previous observations, indicating a trend to increased CEST contrast in more aggressive tumors. While larger studies are needed to validate this result, it indicates the potential of CEST MRI to provide important information on the molecular level that could complement and improve specificity of current breast MRI protocols.

Following stratification by sub-types, the largest MTR_{asym} values were observed in hydroxyl and amine ranges. Moreover, the largest difference between ER– IDC vs ER+ IDC, benign and normal values was observed in hydroxyl MTR_{asym} (Fig. 6), and the strongest correlation with Ki-67 was also observed in hydroxyl and amine ranges (Fig. 7). Previous studies did not differentiate by tumor types, but Ref. (11) had also demonstrated increased hydroxyl MTR_{asym} in malignancy, in general agreement with our observations (Supporting Fig. S1).

Moreover, the focus on hydroxyl and amine range is in agreement with recent studies in cells and animal models that also focused on the hydroxyl MTR_{asym} (9,12). The origins of the increased CEST contrast in the hydroxyl range (or in 1.2 – 1.8 ppm range as in (11)) were attributed to the increase in the glycosaminoglycan concentration (13), alterations in mucin (12) or products of choline metabolism (9,11,25). Moreover, our observations seem to suggest an increase in hydroxyl MTR_{asym} associated with more aggressive metabolism as indicated by ER status and Ki-67 (Figs. 6 and 7). This deviates from the animal model observations, where a decrease in hydroxyl MTR_{asym} was associated with more aggressive cancers (9,12). At the same time, the very large hydroxyl MTR_{asym} (13.6%) observed in the mucinous carcinoma case (Patient 2, Table 1) supports the sensitivity of CEST MRI to mucin concentration as suggested previously (12). More studies are required, in cells, animal models and humans to address the origins of the CEST changes in breast malignancy. Figures 6 and 7 suggest that APT signal, which was proven to be the best for monitoring of brain malignancies (26), might be less suitable for the monitoring of malignant alterations in breast. It should be noted, that the purpose of our study here was not to fully address these important questions, but to provide a new tool for the assessment of breast malignancy at 3T.

In this preliminary study, statistical analyses were not performed for Figures 6, 8 and Supporting Figure S1 due to the small the number of the subjects. In Figures 6 and 8, the number of each group is further reduced when the tumors are stratified by ER status. However, the results do reflect the trend of change in the CEST contrast indicating the potential of CEST imaging in tumor characterization. Currently, a study using improved breast CEST imaging protocol on more subjects is ongoing.

Figure 8 is analogous to Figure 6, but using non-Dixon, second echo source images. The influence of fat on the APT signals in normal, benign and ER+ IDC groups is obvious, with the signals becoming negative. The standard deviations also increase in all the three frequency ranges. Figure 6 clearly demonstrates importance of the efficient water-fat separation in the breast CEST studies and advantage of Dixon method in conjunction with CEST.

Application of multi-echo acquisition and Dixon post-processing has another advantage, even in the areas void of fat: it provides embedded B_0 map acquired at the same time as CEST. Careful B_0 correction is essential for accurate CEST mapping, especially at the lower 3T field and for resonances close to water (i.e. hydroxyl). Great effort is dedicated to careful B_0 mapping, however there are unavoidable uncertainties when separate B_0 map is acquired (using gradient echoes or WASSR (27)), associated with subject's motion between scans and changes in hardware temperature and water frequency drift. Dixon method used here eliminated many of the uncertainties and provided perfectly registered, dynamically updated B_0 maps.

Since all the echoes are acquired in the same shot (Fig. 1), the use of multi-echo Dixon method does not add to the total scan time. Our implementation took about 2.5 min, equivalent to the acquisition time of a standard GRE sequence. Moreover, some time was saved by not acquiring a separate B_0 map, which typically takes ~30 s. While motion was not a problem in most of the cases, it could be a problem for lesions close to the chest wall,

as was the case for Patient 7 (Table 1). In such cases breathing-synchronization strategies and motion post-processing could be employed (28).

Fat suppression in breast imaging can be challenging (29). First, B_0 inhomogeneity is large, deteriorating efficacy of the spectral-selective pulses used in selective fat suppression and SPIR methods. Second, B_1 inhomogeneity creates challenges for inversion pulses (such as in SPIR and STIR). Finally, fat signal composed of multiple fat components with different T_1 s, challenging the SPIR and STIR implementation. In comparison, the Dixon method is insensitive to B_0 and B_1 inhomogeneities (30,31). Moreover, combination of CEST with Dixon avoids interferences of the preparation pulses (and their imperfections) with the CEST saturation train and does not add to the total SAR, which could already be high. Thus, there are numerous potential advantages to using Dixon methods with CEST in breast.

Here, we used the simplest MTR_{asym} analysis. Another alternative to fat suppression could be advanced post-processing using multi-Lorentzian (32) or other model-based fittings. Such methods could offer the advantage of quantitative information on different exchanging moieties. However, IP images have to be acquired to ease fitting, or, the influence of the echo timings (15) should be included in the fitting model. Moreover, multi-Lorentzian fitting is model- and pool number dependent and require sophisticated off-line post-processing. In contrast, all human commercial scanners have at least three-echo version of the Dixon acquisition and post-processing implemented (such as the one that was used here). It is also tempting to speculate that the sequence could be used to differentiate lipid artifact stemming from saturation of non-exchanging fat from relayed NOE occurring via dipolar interaction and exchange with water molecules (33).

Small lesions could still be challenging to detect and analyze, as was the case with Patient 8. The partial volume effects pose the same problem for all imaging methods employed in breast, independent of what fat suppression method is used.

Finally, a technical limitation of our study should be noted. We have employed a bilateral imaging protocol, while unilateral may provide better B_1 homogeneity. Also, in this preliminary study we have chosen B_1 field of 1.2 μT . This value was selected based on a rough optimization in two patients, previous brain studies and reports of breast studies in literature (10). Optimal B_1 value depends on the exchange rate and, thus, may depend on which group is chosen for observation. Previous human studies had focused on APT (10,14), however cell and animal studies indicated higher dependence on hydroxyl protons (9,12), as discussed above. Our preliminary results also indicated increased differences in hydroxyl region. Thus, we have decided to use lower power and a more selective pulse. Moreover, total saturation length was 500 ms, which is the longest allowed in standard RF amplifier operating mode. However, it is known that longer RF saturation leads to improved CEST effect. Based on the preliminary results presented here we can conclude that hydroxyl range shows promise to be most sensitive to the important malignant alterations. Thus, we are conducting further optimization of the saturation protocol, in terms of length and RF power, using alternating transmit to achieve prolong saturation (34) (> 500 ms) and using unilateral imaging.

Conclusions

In the present study, the CEST-Dixon method was shown to be promising for CEST MRI in breast at 3T. Water-fat decomposition leads to homogenous fat removal in the water-only images. The more aggressive ER– IDC malignancy displays trend to higher CEST contrast than the less aggressive ER+ IDC, benign and normal tissues. No significant differences were observed between the ER+ IDC, benign and normal groups. Significant correlation between MTR_{asym} and Ki-67 was observed. The hydroxyl range demonstrated highest correlation with malignant alteration in breast, in agreement with previous cell, animal and human studies. While the study is preliminary the results indicate that the CEST imaging using Dixon for water-fat separation may differentiate between more aggressive and less aggressive breast cancer. A larger clinical study is needed to fully validate these observations and investigate the added value of CEST-Dixon in breast MRI imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The schematic of the CEST-Dixon sequence. A 3-point multi-echo Dixon is used for image acquisition following the CEST preparation. G_R: readout gradient. Ec: echo.

Figure 2.

MTR_{asym} at 1.8 ppm (a), 4.2 ppm (b) and 5.5 ppm (c) for water and iopamidol solutions of different pH with and without Dixon. The non-Dixon and Dixon images were the second echo source images (Ec2) and water-only images (W) respectively.

Figure 3.

In vivo Z-spectra without normalization (a–l) and corresponding images (m–o) of a healthy volunteer in 3 types of images: second echo source image (a–d, m), water-only (e–h, n) and fat-only (i–l, o). The Z-spectra were from separate single pixels with different fat fractions: water only (W: a,e,i), water fraction larger than fat ($W>F$: b,f,j), fat fraction larger than water ($F>W$: c,g,k) and fat only (F: d,h,l). Selected single pixels with different fat fractions are shown as colored dots in the water-only image (n) and in the zoomed-in inset in (h): red: W, blue: $W>F$, yellow: $W<F$ and green: F. The arrow in (g) indicates the residual fat signal.

Figure 4.

Amide CEST maps (a,c,e), the corresponding Z-spectra and MTR_{asym} (b,d,f) with (e,f) and without Dixon (a–d), ROI encompassing all fibroglandular tissue in red (g) and corresponding B₀ map (h). The non-Dixon and Dixon images refer to the second echo source image (a) or first echo source image (c) and water-only image (e). The CEST maps are overlaid on the corresponding reference images: using second echo (a), first echo (c) or water-only (e). The ROI is outlined in red on water-only image in (g).

Figure 5. Hydroxyl CEST maps and ROI averaged Z-spectra (blue) and MTR_{asym} (red) for a healthy volunteer (a,b), invasive ductal carcinoma, not otherwise specified (IDC NOS) patient (c,d, Patient 5 in Table 1) and a triple-negative breast cancer (TNBC) patient (e,f, Patient 3 in Table 1). Note different y-scale for Z-spectra (blue) and MTR_{asym} (red). CEST maps in (a,c,e) are overlaid on the reference water-only images. The panels above (a,c,e) show the corresponding ROIs in red: (b) was averaged across the fibroglandular tissues of both breasts; (d,f) were averaged in the tumor areas as indicated by the ROIs. See Methods section for more details on the ROI placement.

Figure 6.
MTR_{asym} averaged in three frequency ranges for normal, benign, ER+ invasive ductal carcinoma (IDC) and ER- IDC groups.

Figure 7.

MTR_{asym} averaged in the hydroxyl (a), amine (b) and amide (c) frequency ranges against Ki-67 level. The R^2 are 0.95, 0.87 and 0.36 for hydroxyl, amine and amide groups respectively.

Figure 8.
MTR_{asym} averaged in three frequency ranges for normal, benign, ER+ invasive ductal carcinoma (IDC) and ER- IDC groups without Dixon (second echo source image).

Table 1

Pathology data and CEST MRI results.

Patient	Diagnosis	Grade ^a	ER	PR	HER2	Ki-67 (%)	MTR _{asym} (%)		
							(1 ppm)	(2 ppm)	(3.5 ppm)
1	IDC	G3	-	1-2%, weak +	-	80-90	5.2 ± 5.0	6.3 ± 4.6	3.3 ± 4.5
2	IMC	G1	+	+	-	20-25	13.6 ± 6.3	8.9 ± 5.9	4.4 ± 5.4
3	IDC	G2	-	-	-	70-80	5.0 ± 2.0	4.0 ± 2.3	2.5 ± 1.8
4	IDC+EPC	G2	+	+	-	2-5	-1.3 ± 1.4	0.2 ± 0.9	-0.8 ± 4.5
5	IDC	G1	+	+	-	5	0.8 ± 2.9	1.8 ± 2.4	1.6 ± 1.5
6	IDC	G2	+	+	-	10	-0.5 ± 5.3	1.8 ± 6.1	-2.5 ± 4.6
7	IDC	G3	-	-	-	> 95	—	—	—
8	IDC	G2	+	+	-	15	—	—	—
9	ADH	—	—	—	—	—	-1.2 ± 1.5	0.7 ± 1.3	-0.5 ± 3.6
10	Fibroadenoma	—	—	—	—	—	0.8 ± 1.7	2.7 ± 1.8	1.6 ± 2.1

^a Nottingham grade.