Original contributions

Magnetic Resonance Imaging (MRI) of hormone-induced breast changes in young premenopausal women☆

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Abstract

Objectives: We conducted a pilot study to identify whether MRI parameters are sensitive to hormone-induced changes in the breast during the natural menstrual cycle and whether changes could also be observed during an oral contraceptive (OC) cycle.

Materials and Methods: The New York University Langone Medical Center Institutional Review Board approved this HIPAA-compliant prospective study. All participants provided written informed consent. Participants were aged 24-31 years. We measured several non-contrast breast MRI parameters during each week of a single menstrual cycle (among 9 women) and OC cycle (among 8 women). Hormones were measured to confirm ovulation and classify menstrual cycle phase among naturally cycling women and to monitor OC compliance among OC users. We investigated how the non-contrast MRI parameters of breast fibroglandular tissue (FGT), apparent diffusion coefficient (ADC), magnetization transfer ratio (MTR), and transverse relaxation time (T2) varied over the natural and the OC cycles.

Results: We observed significant increases in MRI FGT% and ADC in FGT, and longer T2 in FGT in the luteal vs. follicular phase of the menstrual cycle. We did not observe any consistent pattern of change for any of the MRI parameters among women using OCs.

Conclusions: MRI is sensitive to hormone-induced breast tissue changes during the menstrual cycle. Larger studies are needed to assess whether MRI is also sensitive to the effects of exogenous hormones, such as various OC formulations, on the breast tissue of young premenopausal women.

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1. Introduction

Stimulation of breast cell proliferation is thought to be the main mechanism by which sex hormones increase breast cancer risk. One leading hypothesis, the “estrogen plus progestin hypothesis”, proposes that breast cell proliferation is stimulated by estrogen, and that the addition of progestin to estrogen results in an even greater increase in proliferation [1]. The impact of hormones on mammographic density is consistent with their effect on breast cell proliferation. Both breast cell proliferation and mammographic density are greater in postmenopausal women taking estrogen plus progestin hormone therapy [2–4] compared to women taking estrogen alone [2,4,5] and in the luteal phase of the menstrual cycle (high progesterone) compared to the follicular phase (low progesterone) in premenopausal women [6–10]. These observations suggest that non-invasive radiological methods may be useful for assessing the effects of hormonal exposures on the breast, and could potentially predict the impact of hormonal therapies on breast cancer incidence [11].
The percentage of breast tissue that is fibroglandular tissue (FGT) as measured by magnetic resonance imaging (MRI FGT%) has been shown to be highly correlated with mammographic density [12–17]. MRI has the advantages over mammography that it is 3-dimentional, does not entail breast compression or deformation, and that functional and microstructural tissue parameters can be measured in addition to FGT%. Repeat non-contrast MRI is also more acceptable (particularly for young women) than repeat mammograms to assess hormone-induced changes over a short time because MRI does not expose the breast to ionizing radiation.

Two studies have shown increases in MRI FGT% in the second part of the menstrual cycle [18,19], though a third one did not detect variation during the menstrual cycle[20]. Because these studies did not measure sex hormones, the phase of menstrual cycle could only be estimated. No imaging studies have specifically assessed the effects on the breast of the hormonal preparations most used by young women, i.e., oral contraceptives (OCs). Breast cell proliferation has been shown to be higher among women using OCs vs. naturally cycling women in some [21–23], but not all [24–26], studies. Current and recent use of OCs was associated with increased breast cancer risk in the largest meta-analysis of epidemiological studies to date [27]. However, many of the formulations that were commonly used by women in these studies have been replaced by new ones that include new progestins [28,29], reduced doses of estrogen and progestin [30], and varied administration schedules [31]. There are limited data about the effects of these new OC formulations on the breast tissue and breast cancer risk.

The first aim of this study was to examine changes in MRI FGT% according to phase of menstrual cycle, defined by circulating hormone levels and dates of menses. The second aim was to explore whether changes in MRI FGT% are observed during an OC cycle. We expected to observe increases in MRI FGT% in the luteal vs. follicular phase among women with natural menstrual cycles and in the three weeks of active hormone pills vs. the hormone-free week among OC users. Several functional/microstructural MRI parameters, namely the apparent diffusion coefficient

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(ADC), magnetization transfer ratio (MTR), and transverse relaxation time (T2) were evaluated for their potential to characterize physiological breast changes in young women.

2. Materials and methods

The Institutional Review Board at our institution approved this study. The study is compliant with HIPAA regulations. All participants provided written informed consent.

2.1. Study design and participants

Nine premenopausal volunteers with regular menstrual cycles (length 25-35 days) were recruited through advertisement and eight premenopausal women taking OCs were recruited through a gynecology practice at our institution. All participants in the OC group took Ortho-Novum 1/35® (Ortho-McNeil Pharmaceuticals, Inc., Raritan, NJ). We studied a single OC formulation because the type and dose of hormones differ across formulations [29] and may have variable effects on the breast tissue. None of the participants were pregnant, lactating, or using any hormonal treatments in the 6 months prior to the study, except for Ortho-Novum 1/35 by the OC subjects. Other exclusion criteria included a history of breast disease, oophorectomy, previous breast surgery, and contraindications for MRI (e.g., metallic implants and foreign bodies).

2.2. MRI procedure

Four MRI exams were scheduled for women with natural menstrual cycles during cycle days 3-7, 10-14, 17-21, and 24-32 (counting day 1 as the first day of menses). For women using OCs, the MRIs were scheduled on pill days 3-7, 10-14, 17-21, and 24-28 (first active pill day taken as day 1). At each MRI visit, participants donated 20 mL of blood for hormone measurements. Naturally cycling women were contacted after their last visit to obtain the start date of the next menstrual cycle to calculate cycle length.

Bilateral breast MRI without contrast was performed with the subject prone in a 3T system (TIM Trio, Siemens Medical Solutions, Malvern, PA) using a dedicated 7-channel breast coil (In Vivo, Gainesville, FL). Radiology technologists stabilized the breast using side plates built into the coil to minimize motion artifact. The position of stabilizing plates was recorded at the first visit and the same settings were used for the remaining three visits. The detailed MRI protocol is described in Table 1 [32–37].
2.3. Breast segmentation and inter-modality coregistration

We modified a semi-automated computer algorithm described previously [38] to define the boundary of the breast (see upper panels in Fig. 1) from high resolution axial T1-weighted images. We defined the posterior boundary of the breast by visually selecting the posterior border of FGT laterally toward the axilla on the slice that had the most posterior FGT. A B-spline curve-based method was then used to semi-automatically determine the boundary that separates the breast from air and the chest wall on each 2D slice based on the intensity gradient [38]. Operators manually corrected the boundary between the breast and chest wall when necessary. The stack of the 2D segmented breast regions of interest (ROIs) (red area in the upper-right panel of Fig. 1) defined the breast mask and was used to calculate total 3D breast volume (MRI-V).

The high resolution T1-weighted image used for breast segmentation was then coregistered to the gradient echo images acquired with three different TEs for the 3-point Dixon processing (Table 1). We used SPM (University College London, UK) to compute the affine transformation $T$ based on mutual information as the similarity measure. The breast mask was transformed and resampled into the coordinate space of water/fat data using the same transformation procedure.

To maintain within-subject consistency of breast boundaries, we coregistered images across the four visits using SPM non-linear normalization. Using the transformation matrices, the breast mask from the selected visit was re-sliced for the other three visits. This process was repeated for all visits to have four masks per visit (one originally generated for the selected visit and three coregistered from the other three visits). Coregistration procedures were then repeated for the ADC, MTR, and T2 images. Coregistrations were done for the left and the right breast separately.

Water fractions in all voxels within each breast mask were summed to estimate MRI FGT volume. The total MRI FGT volume was divided by MRI-V (and multiplied by 100) to calculate total 3D breast volume (MRI-V).

The intra-batch CVs were 6.8%, 8.2%, 6.9%, and 11.8% for MRI FGT% in fat, MTR in fat, T2 in fat, and T2 in FGT, respectively. We used the averaged values of all four coregistrations for each image for analysis.

Blinded repeat measurements were done for quality control. The coefficient of variation (CV) for our co-registered MRI FGT% measurements performed by a single operator before and after subject repositioning at a single visit (n=4 pairs) was 4%. The inter-operator CV for MRI FGT% was 3% (n=3 participants with 4 paired measurements each).

2.4. Hormone assays

Estradiol (E2) was measured by radioimmunoassay after extraction with ethyl acetate:hexane (3:2) and Celite column partition chromatography [39]. Progesterone (P4) was measured by chemiluminescent immunoassay on the Immulite Analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). Ethinyl estradiol (EE2) and norethindrone (NET) were measured in the samples from women using OCs, by radioimmunoassay after extraction with ethyl acetate:hexane (3:2) [40]. The CVs were estimated from blinded quality control samples. All samples were assayed in a single batch. The intra-batch CVs were 6.8%, 8.2%, 6.9%, and 11.8% for E2, P4, NET and EE2, respectively.

2.5. Menstrual cycle phase determination

Both hormone levels (Supplemental Table 1) and cycle length were used to classify menstrual cycle phase (early follicular, late follicular, early luteal, late luteal). Each woman did not necessarily have an MRI visit during each phase due to the variability in cycle length among women and scheduling difficulties. The early and late luteal phases were defined as 8-14 and 1-7 days, respectively, before the start of the next menstrual period. Levels of P4 ≥2 ng/mL were also required since an absence of elevation in P4 level is indicative of lack of ovulation. The early follicular phase was the first visit after the first day of menses (days 3-7) and the late follicular phase was the last visit before the luteal phase, provided levels of P4 were compatible with the follicular phase, i.e. ≤0.5 ng/mL.
2.6. OC weeks

Participant visits during OC weeks were classified as week 1 for visits on days 1-7 of the pill pack, week 2 for days 8-14, week 3 for days 15-21, and hormone-free week for days 24-28. EE2 and NET were detectable at each active hormone MRI visit, which indicates that participants were taking the OC. Some women had increases in E2 during the active hormone weeks and had detectable levels of EE2 and NET in the hormone-free week, suggesting they may have missed some active pills and were still taking these missed pills during the hormone-free week. However, endogenous P4 levels remained low ($\leq 0.5$ ng/mL) in all women throughout the active hormone weeks, indicating that ovulation was suppressed for all women (Supplemental Table 1).

2.7. Statistical analysis

For non-OC users and for each MRI measurement, we calculated the percent change for each luteal phase visit (early and late) vs. each follicular phase visit (early and late). For example, the MRI FGT% percent change between early luteal vs. early follicular was calculated as follows: [early luteal MRI FGT% – early follicular MRI FGT%]/[early follicular MRI FGT%] * 100. For OC users, the percent changes were calculated for each week of active hormone pills vs. the hormone-free week. We used the paired Wilcoxon signed-rank test to assess whether the percent changes in breast MRI measures between each menstrual cycle phase or between OC weeks were statistically different from zero. All statistical significance levels (p values) are two-sided.

3. Results

Table 2 shows descriptive characteristics of the study participants. The median age at enrollment was 27 years (range 24-31). For naturally cycling women, the cycle length on study was between 25-35 days, BMI was within the normal range (20-24 kg/m\(^2\)), and none of the women had ever been pregnant. OC users had been using Ortho-Novum 1/35 for 1-7 months prior to the study, had BMI ranging from 19-29 kg/m\(^2\), and 50% had had at least one pregnancy. MRI parameters for the reference week (early follicular phase or OC hormone-free week) are also shown in Table 2. The differences between OC users and naturally cycling women (e.g. higher BMI, more likely to be parous, larger MRI-V, and lower MRI FGT%), although not unexpected given the different recruitment sources, prevented us from directly comparing MRI parameters between these two groups.

3.1. Naturally cycling women

Fig. 2 shows the relative changes in MRI total volume (MRI-V), MRI FGT volume, and MRI FGT% measurements. For each phase comparison there are less than 9
women because each woman did not necessarily have an MRI visit during each menstrual cycle phase. Most women had increases in FGT volume and FGT% in the luteal vs. the follicular phases (Figs. 2B, 2C, and 2D), but not in the late follicular vs. early follicular phase (Fig. 2A). The late luteal versus early follicular phase increase in MRI FGT% was significantly different from zero (median relative increase: 4.9%, range: -4%–6.7%, P = .047).

Fig. 3 shows the relative changes in ADC, MTR, and T2 measures. The ADC in FGT was significantly higher for the late luteal vs. late follicular (median relative increase: 14%, range: 6%–22%, P = .03, Fig. 3D) and early luteal vs. late follicular (median increase: 9%, range: 2%–39%, P = .03, data not shown) phases. T2 in FGT was significantly longer in the late luteal vs. late follicular phase (median increase: 6%, range: 0.1%–17%, P = .03). There was no significant pattern of change for MTR in FGT or T2 in fat across the menstrual cycle. Though most women had large increases in ADC in fat, and some had increases in MTR in fat, in the luteal vs. follicular phase of the menstrual cycle, these increases were not statistically significant.

3.2. OC users

Fig. 4 shows the relative changes in MRI total volume (MRI-V), MRI FGT volume, and MRI FGT% and Fig. 5 the relative changes in ADC, MTR and T2 for each week of active hormone pills vs. the hormone-free week. No consistent pattern was observed for any of these parameters, although some women exhibited increases or decreases of up to 12% in MRI FGT volume and 11% in MRI FGT%, over 20% for ADC in fat and FGT and over 10% in MTR and T2 in the active hormone weeks vs. the hormone-free week.

4. Discussion

The range of values we observed for total volume (MRI-V), MRI FGT volume, and MRI FGT% was consistent with those reported in a large study of young healthy women [16]. We found significant increases in MRI FGT% and higher ADC and T2 in FGT between the follicular and luteal phases of the menstrual cycle. Among women using OCs, there were no consistent patterns through the OC cycle.

Studies of breast changes at the cellular level have shown that proliferation increases 2-3 fold in the luteal vs. follicular phase of the menstrual cycle [9,10]. Thus, the increase in MRI FGT% we observed in the luteal phase may be reflective of increased proliferation. Our finding is consistent with two previous studies that observed an increase in MRI FGT% during the second half of the menstrual cycle [18,19]. Although a recent, larger study did not observe variation in MRI FGT% during the menstrual cycle, it was limited to slim Asian women [20]. A limitation of these previous studies,
however, is that menstrual cycle phase was estimated based on self-report of menstrual cycle length, which cannot be used to reliably distinguish ovulatory vs. non-ovulatory cycles or timing of cycle phases due to the substantial variability between- and within-women in menstrual cycle characteristics [41,42]. The hormonal measurements in our study confirmed that all cycles were ovulatory, reflected the expected hormonal changes known to occur during the menstrual cycle (lowest E2 and P4 during menses, increases in E2 during the follicular phase, and increases in P4 in the luteal phase), and allowed us to assign phase of cycle with confidence. Our results provide further support that the elevated levels of P4 in the luteal phase are accompanied by MRI FGT%.

Our finding that ADC in FGT increased in the luteal phase of the menstrual cycle is consistent with the findings of Partridge et al. [43], who proposed that this increase reflects the increases in secretion activity, stromal edema, and water volume in the extracellular matrix known to occur during the luteal phase [43]. We also observed increases in T2 in FGT in the luteal vs. follicular phase of the menstrual cycle, which may reflect the increasing water content of the breast towards the end of the cycle. This result is consistent with the findings of one previous study (n=8 women) [44], although two studies (n=3-5 women) did not observe changes in T2 [45,46].

Previous studies were based on small ROIs that were manually selected on a single 2D slice which raises the concern of subjectivity [43–46]. To avoid this concern, we performed measurements of ADC and T2 in an automatically defined 3D region. We also selected the ROI to only include tissue with >75% FGT or >75% fat which minimized the partial volume effect.

Although natural estrogens and progesterone are low throughout the OC cycle, we expected to see changes in breast tissue in the active-hormone weeks vs. hormone-free week because of the exposure to the exogenous estrogen and progestin contained in the pill. We did not observe, though, any significant difference in any of the parameters we examined. Although P4 was suppressed for all women, indicating that women were taking the pill sufficiently to suppress ovulation, EE2 and NET were detected for some women during the hormone-free week. Missed pills are common [47], and if some women were behind schedule, they may have still been taking active hormone pills during the scheduled hormone-free week. The lack of a clear hormone-free reference week may explain why we did not observe any pattern of change among OC users. We
performed analyses excluding OC users that had high E2 levels (>100 pg/mL) during the active hormone weeks and also excluding women with detectable levels of NET and EE2 in the hormone-free week. These sensitivity analyses gave results similar to those of our analyses of all OC users, i.e., we did not observe any pattern of FGT% change during the OC cycle. In addition, our sample size was small. We note that due to the large number of parameters evaluated some of our results may be chance findings. We did not use statistical methods to adjust for multiple comparisons because this pilot study was not designed to statistically test specific hypotheses but rather to obtain estimates of MRI parameters and their variability for the planning of future, larger studies. Further, we studied only one OC formulation; other formulations, in particular those that may be associated with increased breast cell proliferation and breast cancer risk, may have a larger effect [22,48]. Finally, the effects of OCs on the breast might be more apparent in studies comparing MRIs during OC use to MRIs during the natural cycle in the same women.

In summary, we observed increasing MRI FGT% and higher ADC and T2 in FGT in the luteal vs. follicular phase of the menstrual cycle, suggesting that FGT%, ADC, and T2 may be of interest in future MRI studies examining the effects of hormonal exposures on the breast tissue. We did not observe a pattern of breast tissue changes during the OC cycle, which may be due to limited compliance. Larger studies are needed to assess the potential effects of various OC formulations on the breast tissue.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mri.2012.06.022.

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