# Are There Differences in Breast Tissue as a Result of Hormone Replacement Therapy? Can BEST Imaging Distinguish These Differences?

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**Although it has been speculated that estrogen therapy may promote changes in breast tissue that could lead to cancer, no information exists as to differences in breast tissue for women who do and do not take hormone replacement (HRT) therapy. This study seeks to determine if there are differences in the tissue of women taking HRT in contrast to those who do not and if these differences are apparent in cases of breast cancer, cellular atypia, fibrocystic (FCD) disease and normal breasts. A total of 327 non-pregnant, nonlactating, pre-menopausal women were enrolled in the study, including 139 women who were actively taking HRT and 188 women who never had taken HRT. Using breast enhanced scintigraphy test (BEST) imaging, differentiation of breast tissue was determined. The groups were then analyzed to determine the effect of hormone therapy within each category of breast tissue. Differentiation between normal, FCD, cellular atypia, and breast cancer represent statistically significant differences (p.001) in metabolic activity and vascularity as demonstrated by differences in both average count activity (ACA) and maximal count activity (MCA). The distinction between cellular atypia and infiltrating breast cancer was statistically (p.05) different when looking at the maximal activity. Normal breast tissue and breasts with FCD appear more homogenous with no statistical differences in variability in breast tissue. Tissue variability is statistically greater when localized processes, such as cellular atypia and breast cancer, are present. Differentiation of cellular metabolic activity in breast tissue can be statistically determined when looking at the average and maximal metabolic activity. The final distinction between cellular atypia and cancer occurs when a focal region of breast tissue becomes metabolically more active than the surrounding breast tissue as shown by statistical increases in MCA. These findings are confirmed by the increased metabolic variability seen in regions of cellular atypia and cancer compared with the homogenous metabolic activity present in normal and fibrocystic breasts.**

*Keywords: Hormone replacement therapy (HRT); breast cancer; breast imaging; breast-enhanced scintigraphy test (BEST)*

The ability to distinguish between normal breast tissue and breast cancer using anatomic methods is limited by the physics of the instrumentation used, for example, radiographs (mammography), ultrasound, computed tomography, and magnetic resonance imaging. Like heart disease, breast cancer does not suddenly appear from normal breast tissue. Changes in cellular metabolism and promotion of increased vascularity (angiogenesis) must occur as normal breast cells mutate into cancer cells. Evaluation of the transition in anatomic change is limited by an inability to evaluate these metabolic changes. Nuclear imaging of the breast using isotopes can be used to look at the metabolic function of cells by employing isotopes that are taken up by the mitochondria[1-6] of breast cells and by detecting changes in blood flow[6,7] present with increased vascularity resulting from vascular endothelial growth factors. Such approaches can even be used to determine the effectiveness of chemotherapy[8] treatment.

These prior attempts to detect changes in breast tissue[9,10] have been limited by the ability to change blood flow and subsequent isotope uptake into mitochondrial tissue. This limitation was overcome with the development of breast-enhanced scintigraphy test (BEST) imaging,[11-13] which takes advantage of evaluating both mitochondrial activity and changes in blood flow. Thus, BEST imaging has been shown to differentiate between normal breast tissue, fibrocystic (FCD) disease, cellular atypia, and breast cancer.

Our initial observations[11-13] using BEST imaging suggested that women taking HRT had greater tissue variability than women who did not. Presumably the use of estrogen can promote localized changes in breast tissue and increase both metabolic (mitochondrial) changes within breast tissue and vascularity within the breast. In this study, 327 pre- and

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perimenopausal women were studied to determine if they had breast cancer, cellular atypia, FCD, or normal breasts. Use of HRT by these women was then assessed. The BEST findings were compared among different pathological groups and between HRT users and nonusers, and they are presented here. The information obtained also confirm prior research findings with BEST imaging and enhance the understanding of metabolic changes seen on BEST imaging among women taking and not taking HRT.

## Methods

*Patient Enrollment.* Three hundred twenty-seven nonpregnant, nonlactating pre- and perimenopausal women were enrolled in the study after an initial evaluation by BEST imaging. The women ranged in age from 30 to 60 years of age. Those taking HRT were using both estrogen and progesterone and were receiving HRT for birth control, for postoophorectomy with treatment focus on osteoporosis, and for hot flashes and related perimenopausal symptoms. Participation in the study required that the women were either currently taking hormone therapy or had never taken hormone therapy. Patients included in the study had not previously been diagnosed with or treated for breast cancer. Individuals who had previously taken HRT but had stopped were excluded from the study. The study was approved by the relevant institutional review board, and all participants signed institutionally approved informed consent forms prior to participation in the study.

*BEST Imaging.* Each woman underwent breast imaging[11-13] as described previously. Images acquired from BEST were analyzed to determine the total count activity present and the number of pixels involved. The average and maximal/greatest uptake of sestamibi by breast tissue were calculated as the average number of counts per pixel for the image as a whole (average count activity or ACA), and the number of counts per pixel for areas of concern as determined by the physician trained in nuclear imaging or the radiologist (maximal count activity or MCA). Standard deviations for both ACA and MCA were also determined for each. The ACA represented the overall state of breast tissue metabolic activity. The MCA represented the most metabolically active component of breast tissue.

*Pathology.* Histopathologic specimens were obtained in all cases of breast cancer and cellular atypia to determine estrogen receptor status.

*Statistical Analysis.* The average and standard deviation were determined for each study along with the MCA and the associated standard deviation. Differences between groups were determined using Fisher's 2-tailed *t* test. Tissue variance was determined from standard deviation data and analyzed for differences using *F* ratio. Differences were determined to be statistically significant if and only if the *P* values were less than .05 as commonly defined.[14]

## Results

Of the 327 women enrolled in the study, 42.5% (139 of 327) were taking HRT and 57.5% (188 of 327) were not and had never taken hormone therapy. Table 1 shows both the ACA ($\overline{X} \pm \sigma$) plus or minus the standard deviation, representing overall metabolic activity of breast tissue, and the MCA (MCA $\pm \sigma$) plus or minus the standard deviation, representing the areas of greatest metabolic activity present in the breast. Both the ACA and MCA increased with the progression from normal to FCD to cellular atypia to breast cancer; these states demonstrate increasing metabolic activity (mitochondrial activity) and vascularity (angiogenesis), as previously published.[11-13] For the 141 women with normal breast tissue, the mean ACA value was 95  26 with a mean MCA of 206 ± 54. Metabolic activity was higher in the 139 women with FCD, with a mean ACA value of 115 ± 30 and a mean MCA of 250 ± 57. As shown in Tables 2A and 2B, the levels of the ACA and MCA values were significantly different, $P < .001$ and $P < .001$ respectively, in women with FCD and those with normal breasts.

Women with cellular atypia represented 10.4% (34 of 327) of the women who participated in the study. The mean ACA result was 140 ± 38, whereas the mean MCA was 320 ± 100. As shown in Tables 2A and 2B, the ACA and MCA results were significantly different ($P < .001$ and $P < .001$, respectively) in this group and in women with FCD. Women with breast cancer showed further elevations in metabolic activity, with a mean ACA value of 189 ± 118 and a mean MCA of 429 ± 159. The ACA value, although greater than that seen for cellular atypia, is not statistically different. The MCA is, as shown in Table 2B, statistically different ($P < .05$) from that seen with cellular atypia. The results of the average and MCA results are graphically depicted in Figures 1 and 2.

Table 3 defines differences in tissue variability when analyzed using the standard deviations of ACA and MCA shown in Table 1. There is a slight difference in tissue variance for both the ACA and MCA findings when normal breast tissue is compared to FCD. These differences, however, are minor and not statistically

**Table 1. Average Count Activity (ACA) and Maximal Count Activity (MCA) Results of BEST Imaging in 327 Women**

| *Category* | $\overline{X} \pm \sigma$ | *MCA* $\pm \sigma$ | *Number of Women* |
|---|---|---|---|
| Normal | 95.27 ± 25.85 | 205.93 ±54.30 | 141 |
| Fibrocystic disease | 114.58 ± 29.85 | 249.68 ± 57.41 | 139 |
| Atypia | 139.77 ± 38.07 | 320.1 ± 100.6 | 34 |
| Cancer | 189.4 ± 117.6 | 429.0 ± 159.0 | 13 |

$\overline{X} \pm \sigma$ indicates mean ACA ± the standard deviation (counts per pixel). MCA ± σ indicates mean MCA ± the standard deviation (counts per pixel).

**Table 2A. Statistical Differences in the Average Count Activity (ACA)**

| *Category* | *Normal* | *Fibrocystic Disease* | *Atypia* |
|---|---|---|---|
| Normal | NA | | |
| Fibrocystic disease | *P* < .001 | NA | |
| Atypia | *P* < .001 | *P* < .001 | NA |
| Cancer | *P* < .005 | *P* < .025 | *P* = NS |

NA = not applicable.

**Table 2B. Statistical Differences in Maximal Count Activity (MCA)**

| *Category* | *Normal* | *Fibrocystic Disease* | *Atypia* |
|---|---|---|---|
| Normal | NA | | |
| Fibrocystic disease | *P* < .001 | NA | |
| Atypia | *P* < .001 | *P* < .001 | NA |
| Cancer | *P* < .001 | *P* < .001 | *P* < .05 |

NA = not applicable.

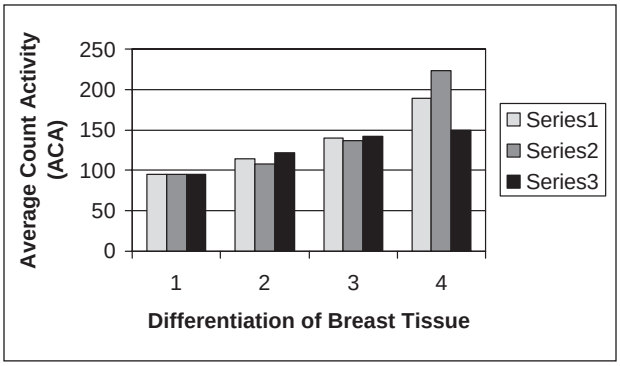


*Figure 1 Differences between women receiving hormone replacement therapy (HRT) and those who did not. The x-axis shows the results of tissue differentiation as confirmed by both nuclear imaging and biopsy results. Breast differentiation was defined as normal (1), fibrocystic disease (2), cellular atypia (3), and carcinoma (4) of the breast. The average count activity (ACA) on nuclear imaging of these women is shown on the y-axis. A total of 327 women were studied during a 4-year period of time and are represented by series 1. Series 2 represents 188 (57.5%) of these women who did not use HRT. Series 3 represents the findings of the 139 (42.5%) women who were taking HRT. The findings, reported in Tables 1 and 4, reveal an increased uptake in isotope (mitochondrial) activity as increased pathological change in breast tissue was seen. The count activity is greater in almost all groups among women taking HRT than among those who have not. This difference is statistically greater in women with fibrocystic disease.*

significant. A further elevation in tissue variability is seen when comparing the results of FCD and cellular atypia. These differences are statistically ($P < .05$) significant, as are the differences in metabolic variability ($P < .01$) between cancer and FCD. The difference in tissue metabolic variability between cellular atypia and breast cancer is not statistically significant.

The women were then subdivided into groups of those who took HRT (139) and those who had never taken hormone therapy (188). As shown in Table 4, there were 141 women with normal breast tissue, of whom 66.7% (94 of 141) had never taken HRT and 33.3% (47 of 141) took HRT. The ACA and MCA assessments of metabolic activity were similar ($P = NS$) regardless of whether the women were taking hormones. Figures 1 and 2 represent the bar graph findings for the ACA and MCA results, respectively.

When women were subdivided according to the use of hormone therapy, women with FCD who were not taking HRT had a statistically lower ACA ($P < .01$) and MCA ($P < .001$) than did women taking HRT. Of the women with cellular atypia (55.9%, 19 of 34) and cancer (46.2%, 6 of 13), there were a disproportionately high number of women taking HRT. There were no statistical differences in ACA or MCA between women with cellular atypia or those with cancer based upon their use of hormone therapy. Two (15%) of the 13 cancers were estrogen receptor negative. Both of these women were in the non-hormone-treatment group. The women with ER-negative tumors accounted for the higher ACA and MCA results seen in the no-HRT treatment group of women with cancer. The ER-negative cancers had greater ACA and MCA values, suggesting possible differences in vascularity and mitochondria among ER-negative and ER-positive breast cancers. Figures 1 and 2 show the average and MCA values for women taking hormone therapy (series 3), those who did not (series 2), and all the women in the study (series 1).

Finally, in Table 5 tissue variability was again analyzed looking at women who had never taken hormone therapy and those taking HRT. In both the no-HRT and the HRT groups, there were statistically significant differences in tissue variance between women

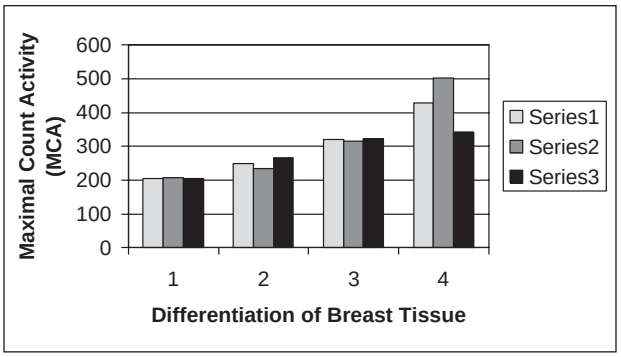


*Figure 2 Differences in maximal count activity (MCA) associated with differentiation of breast tissue and the presence or absence of HRT. The x-axis distinguishes breast tissue changes as discussed in Figure 1 compared with the maximal count activity (MCA) present in breast tissue. The differentiation of breast findings and groups represented are identical to that defined in Figure 1. The results of this graph reveal an increased MCA between breast tissue that is normal, to fibrocystic changes, to cellular atypia, to breast cancer. The maximal count activity is greater in almost all groups for women taking HRT than in those who do not. This difference is statistically significant among women with fibrocystic disease of the breast.*

who had cellular atypia or breast cancer and those with normal breasts or FCD. There were, thus, no differences in patterns of tissue metabolic variability of different pathological states seen in women related to their use of hormone therapy. Thus, if tissue variability is used to distinguish cellular atypia or breast cancer from normal tissue or FCD, the use of HRT is not expected to be a confounding factor.

## Discussion

Evaluation of women with BEST imaging demonstrates increased metabolic activity when normal breast tissue is compared with FCD and when FCD is compared with cellular atypia. When breast cancer is compared with the findings of cellular atypia, the ACA values, although greater, are not significantly different, suggesting less differentiation between the metabolic activity of cancerous breast tissue and cellular atypia than previously thought. These findings further suggest that cellular atypia might be more correctly thought of as precancers or early cancers. The MCA was statistically different between cellular atypia and infiltrating breast cancer. Since MCA is a marker for the most metabolically active region of the breast, this statistical difference between cellular atypia and infiltrating cancer of the breast is a direct reflection of a focal region of breast tissue that has made the final transition to fulminant breast cancer.

As evidenced by the standard deviations ($\sigma$) of the ACAs and MCAs in Table 1, there is an increase in $\sigma$ as the metabolic activity of breast tissue increases. The variance ($\sigma^2$) or variability of breast tissue increased as metabolic activity increased. The findings showed statistical increases in breast tissue variability between normal breast tissue and those with cellular atypia and cancer. There was no statistical difference in the variability of breast tissue in women with normal breasts and those with FCD. The reason for this appears fundamentally obvious. Women with normal breasts have no localized hypermetabolic region of the breast. Women with FCD have a diffuse process affecting most if not all of the breast tissue, leaving little variability between regions of the breast. In contrast, women with cellular atypia and infiltrating breast cancer have localized processes that are metabolically distinct from surrounding normal or even regions of FCD breast tissue. As such, this area of significantly higher activity is metabolically more active than the surrounding homogenous breast tissue.

**Table 3. Statistical Differences in Variability (Regional Differences) in Breast Tissue**

| *Category* | *Normal* | *Fibrocystic Disease* | *Atypia* |
|---|---|---|---|
| Normal | NA | | |
| Fibrocystic disease | $P$ = NS | NA | |
| Atypia | $P$ = NS | $P$ < .05 | NA |
| Cancer | $P$ < .01 | $P$ < .01 | $P$ = NS |

NA = not applicable.

Women using HRT who had FCD had statistically greater metabolic activity in both the ACA ($P$ < .01) and the MCA ($P$ < .001) values, when compared with women who did not take hormone therapy. The initial change seen among women taking HRT is an increase in tissue variability, which is reflected in an increase in MCA, with ACA remaining in the FCD range. Thus, BEST performed on women with FCD who take HRT could cause confusion with cellular atypia if only MCA values are assessed. This increased MCA would be due to localized regions of cellular change, placing them further along the road toward cellular atypia/breast cancer than the non-HRT women with FCD who have lower MCAs. Of the 7 breast cancers in the group of women who were not taking HRT, 2 of these were ER-negative. These women had higher ACA and MCA values and as such resulted in greater ACA and MCA values for the group of women not taking HRT. As such, they presumably are more aggressive in growth and subsequently more metabolically active accounting for the nonsignificantly greater ACA and MCA values seen in this group of women relative to cancer patients taking HRT. There were no significant differences in the metabolic activity of women with normal breasts related to their use of hormone therapy.

There were no significant differences in the metabolic activity seen in women taking HRT with breast cancer and those with breast cancer who do not take

**Table 4. Average Count Activity (ACA) and Maximal Count Activity (MCA) Results of BEST Imaging in 327 Women, Including 188 Not Taking Hormone Replacement Therapy (HRT) and 139 Taking HRT**

| *Category* | $\bar{X} \pm \sigma$ | *MCA* $\pm \sigma$ | *Number of Women* |
|---|---|---|---|
| Normal (all) | 95.27 ± 25.85 | 205.93 ± 54.30 | 141 |
| Normal (–HRT) | 95.21 ± 22.00 | 206.74 ± 51.15 | 94 |
| Normal (+HRT) | 95.38 ± 32.48 | 204.30 ± 60.66 | 47 |
| Fibrocystic disease (all) | 114.58 ± 29.85 | 249.68 ± 57.41 | 139 |
| Fibrocystic disease (–HRT) | 108.38 ± 32.76 | 233.88 ± 56.16 | 72 |
| Fibrocystic disease (+HRT) | 121.26 ± 24.93 | 266.67 ± 54.16 | 67 |
| Atypia (all) | 139.77 ± 38.07 | 320.1 ± 100.6 | 34 |
| Atypia (–HRT) | 137.09 ± 34.54 | 316.5 ± 94.3 | 15 |
| Atypia (+HRT) | 141.88 ± 41.46 | 322.80 ± 107.80 | 19 |
| Cancer (all) | 189.4 ± 117.6 | 429.0 ± 159.0 | 13 |
| Cancer (–HRT) | 223.4 ± 148.2 | 503.0 ± 137.9 | 7 |
| Cancer (+HRT) | 149.7 ± 57.5 | 342.4 ± 145.6 | 6 |

$\bar{X} \pm \sigma$ indicates average count activity (ACA) ± the standard deviation (counts per pixel). MCA ± σ indicates maximal count activity (MCA) ± the standard deviation (counts per pixel).

**Table 5. Statistical Differences in Variability (Regional Differences) in Breast Tissue in Women Taking Hormone Replacement Therapy (HRT) and Those Not Taking HRT**

| | *–HRT* | | | *+HRT* | | |
|---|---|---|---|---|---|---|
| *Category* | *Normal* | *Fibrocystic Disease* | *Atypia* | *Normal* | *Fibrocystic Disease* | *Atypia* |
| Normal | NA | | | NA | | |
| Fibrocystic disease | *P* = NS | NA | | *P* = NS | NA | |
| Atypia | *P* < .01 | *P* < .01 | NA | *P* < .01 | *P* < .01 | NA |
| Cancer | *P* < .01 | *P* < .01 | *P* = NS | *P* < .05 | *P* < .01 | *P* = NS |

NA = not applicable.

hormone therapy. The same was true when cellular atypia was present. This is to be expected since the presence of cellular atypia and/or breast cancer would produce the expected change in metabolic activity regardless of whether hormone therapy was being used or not. That is, the change in variability and loss of homogeneity is a marker of cancer, not the presence or absence of estrogen. In fact, the presence of estrogen could potentially nullify differences in breast tissue by increasing metabolic activity in the remainder of breast tissue, thereby reducing the ability to detect differences in regions of increased metabolic activity as a consequence of increasing metabolic activity throughout the remainder of the breast.

## Conclusions

Differences in metabolic activity (including both mitochondrial and angiogenic) are statistically different in regions of cancer, cellular atypia, FCD, and normal breast tissue. The differences are reflected in overall increases in metabolic activity, but more important, in localized regions of increased metabolic activity as evidenced by higher MCA and tissue variance in cellular atypia and cancer versus the homogeneity seen in normal and fibrocystic breasts. Women who took HRT had greater metabolic activity when they had FCD than did women who did not use hormones. The prevalence of breast cancer and cellular atypia was higher among women who took HRT, but once breast cancer or cellular atypia occurs, there are no statistical differences to distinguish between groups. The metabolic activity was greater for the ER-negative tumors than the ER-positive ones. Further investigation should include information about interleukin values since IL-8 is associated with tumor invasiveness[15] and follows our predicted change of cancer metabolism.[16] Tissue metabolic variability was a factor of cellular atypia and/or breast cancer. This higher metabolic activity is partially masked by the use of HRT, which provides metabolic stimulation to the normal or FCD tissue, thereby lessening differences between that seen in estrogen-stimulated tissue and the more metabolically active atypia and cancer. This information suggests that BEST imaging can be improved upon by looking at ACA, MCA, tissue variability, and use of HRT in individual patients.

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