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Obesity is Associated with Atypia in Breast Ductal Lavage of Women with Proliferative Breast Disease

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Abstract

Background—Benign proliferative breast disease without atypia slightly increases breast cancer risk but there are currently few clinical options for breast cancer prevention in this group of women.

Methods—We conducted a pilot study of women with a past diagnosis of proliferative breast disease with a goal to determine if the characteristics of cells obtained by breast ductal lavage were related to nutritional factors.

Results—There were 57 women who enrolled. A total of 39 women yielded nipple aspirate fluid (NAF) samples and 36 underwent breast ductal lavage. Five of the lavage samples were acellular and 28 had at least 200 cells. Surprisingly, atypia was present in 11 women. Presence of atypia was associated with slight changes in morphometric features of the epithelial cells such as measures of circularity) as obtained by image analysis, but the only variable significantly different in women with atypia (versus no atypia) was a higher mean body mass index. Body mass index also was significantly correlated with C-reactive protein (CRP) levels in the nipple aspirate fluid, indicating that obesity might have a pro-inflammatory effect on the breast that can contribute to increased rates of atypia.

Conclusions—Although the clinical significance of atypia in breast ductal lavage is uncertain, these results support further work on prevention of obesity as a strategy for reducing breast cancer risk.

Keywords

proliferative breast disease; nuclear morphometry; breast ductal lavage; obesity

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1. Introduction

Benign breast disease is a common finding upon breast biopsy. Many conditions do not significantly increase breast cancer risk, but proliferative breast changes, without atypia, are associated with a reported increase in breast cancer risk that has ranged from 1.3 to 2.2 [1-4]. Fibrocystic changes in general have been hypothesized to be part of the sequence going from normal breast epithelium to carcinoma, and fibrocystic disease that is proliferative has a higher frequency of chromosome aberrations versus non-proliferative disease [5,6]. In the MCF-10A model of human breast carcinogenesis, proliferative changes are observed early in the transformation process [7]. Women with proliferative change in the breast may therefore appear to be an appropriate population for prevention using lifestyle approaches.

From a practical standpoint, women with proliferative disease could be good candidates for clinical assessment using breast ductal lavage since this condition should result in a high percentage of women yielding epithelial cells in the lavage fluid. In the normal breast, obtaining epithelial cells from biopsy specimens is very difficult due to strong cell-cell adhesions. In proliferative lesions, epithelial cells dislodge more easily and women with proliferative breast disease have relatively increased epithelial cell proportions in their breasts [8,9]. In a study of nipple aspirate fluid (NAF) specimens, the NAF from women with symptoms of benign breast disease contained more than twice as many cells as that from women with normal breasts [10].

Proliferative breast disease appears to result from hormonal or genetic factors, and is likely to affect both breasts. In fine needle aspirates of clinically normal breasts, proliferative breast disease was detected in 35% women who had at least two first degree relatives with breast cancer, while only 13% of women with no such family history had this diagnosis [11]. Use of postmenopausal estrogens was associated with increased risk of proliferative breast disease in the Women's Health Initiative trial [12]. There are scant data in the literature about whether breast cancer risk subsequent to surgical removal of proliferative regions without atypia is the same in both breasts. In a long-term study of women with atypical ductal hyperplasia, 56% of the subsequent breast cancers were in the ipsilateral breast. For women with atypical lobular hyperplasia, 69% of those cancers were in the ipsilateral breast [13]. In the Nurse's Health Study, breast cancer after a diagnosis of atypical hyperplasia developed in the ipsilateral breast only 59% of the time [14]. This indicates that presence of proliferative changes in one breast can be regarded as a risk factor for breast cancer in either breast, and the changes are likely not confined to the excised lesion.

An important goal of this exploratory study of breast ductal lavage in women with proliferative breast disease was to gain preliminary feasibility data for intervention studies of diet and/or weight control. A diagnosis of proliferative breast disease is common upon breast biopsy of suspicious lesions, yet little beyond surgery is offered to these women. Reports of the influence of diet, alcohol intake and smoking on risk of benign breast disease are encouraging in that lifestyle changes might be useful to reduce risk [15-19]. Morphological characteristics of the breast epithelial cells may be a sensitive endpoint for such interventions. A prospective study on follow-up of women with either normal biopsy or usual hyperplasia indicated an increased subsequent breast cancer risk for those in the highest 25th percentile for nuclear area and perimeter in the breast epithelial cells [20]. This study therefore explored whether dietary factors and body mass index (BMI) are associated the morphological characteristics of the breast epithelial cells obtained from ductal lavage of women with proliferative breast disease.

2. Methods

2.1 Recruitment of subjects and eligibility criteria

The study was approved by the Institutional Review Boards of Wayne State University and the University of Michigan (clinical trial registration NCT00259597). Enrollment in the study occurred in the Detroit, MI metropolitan area from 5/17/05 through 6/14/07 with the exception of three subjects enrolled in July of 2004, due to a change in institutions by the study director. The major source of recruitment was direct invitation of potentially eligible women at the Karmanos Cancer Institute's Walt Comprehensive Breast Clinic. Other sources included flyer distribution at health fairs, and direct mail letters to women who were members of a Benign Breast Disease cohort established by Dr. Maria Worsham at Henry Ford Hospital. Subjects gave written, informed consent to participate, and they were provided with parking passes and \$75 cash for their time and inconvenience related to study participation.

Eligible women had a previous diagnosis of proliferate breast disease without atypia from a breast biopsy. Clinical information regarding past biopsies was obtained from hospital records. Proliferative breast disease was defined as moderate or florid hyperplasia, papilloma, sclerosing adenosis, or radial scar. Other eligibility criteria were: age 18 and older, at least 1 month post biopsy, at least 3 months post any preventive treatments such as tamoxifen, not pregnant, at least 3 months post lactation, and be in good general health. Persons with diabetes or uncontrolled high blood pressure were excluded.

2.2 Clinical procedures

Women were asked to complete questionnaires, be measured for height and weight, provide a blood sample and undergo breast ductal lavage. Weight was obtained to the nearest pound in light clothing using a professional beam scale. Height was obtained to the nearest 0.5 inch using a stadiometer. There were two questionnaires used: a Health Status Questionnaire that included reproductive history, health and cancer history, and the Block'98 Food Frequency Questionnaire (Nutritionquest, Berkeley, CA). The Block'98 Food Frequency Questionnaire has performed well versus food records for assessment of usual dietary intakes in our previous study of women at increased breast cancer risk [21]. This questionnaire was first developed using a population sample in the suburban Detroit area (Ypsilanti, MI) that included African Americans [22]. The Health Status Questionnaire was used to calculate breast cancer risk using the Gail model with an online calculator provided by the National Cancer Institute [23]. This version of the calculator did take into account race. Exercise was assessed by asking how many hours per week the woman "exercised or was physically active in a typical week."

After women completed the questionnaires, they were asked to place Ela-Max cream with a Tegaderm shield (AmericaRx.com) on their breasts and to watch a brief video on the ductal lavage procedure supplied by Pro-Duct Health (Menlo Park, CA). The nipple was then cleaned and they were instructed to start breast self-massage. A nurse practitioner continued the massage, if needed, until NAF was produced. The emphasis was on obtaining NAF and performing lavage on the breast ipsilateral to the previous biopsy: if that was not possible, an attempt was made on the contralateral breast. The NAF was collected in a heparinized capillary tube. If it was not possible to obtain NAF, ductal lavage was still attempted when a duct could be located.

A flexible Pro-Duct micro-catheter was used to flush catheterized ducts with 20-ml lavage fluid, using 1% lidocaine anesthetic in the fluid to prevent discomfort. One duct per woman was lavaged with the exception of one woman on whom both breasts were lavaged. For this woman, one breast had benign cells and the other breast had atypical cells. The breast with

atypical cells was used in the statistical analyses shown in the tables. For any woman on whom atypia was noted in the breast epithelial cells, a letter was sent to the woman's physician to communicate this finding and to suggest possible clinical follow-up options.

2.3 Sample processing and analysis

The ductal lavage fluid was collected in a 50-ml plastic tube. Both the lavage fluid and NAF were placed on wet ice and processed within 30 minutes of the procedure. The lavage fluid was centrifuged at 300g for 10 minutes, the supernatant frozen and the collected cells were fixed in PreservCyt (Cytoc Corp., Boxborough, Massachusetts). Slides were made using the Cytospin method and Papanicolaou staining in the clinical cytopathology laboratory. The NAF collected in capillary tubes was rinsed into an Eppendorf tube by flushing the capillary with 50 μ l of 5% USP dextrose containing 50 mM mannitol –10 mM Tris, pH 7.4. It was frozen at –80°C until analysis. Plasma was prepared from blood in heparinized tubes and aliquots frozen at –80°C.

The plasma was assayed for carotenoids and fatty acids using published methods. Briefly, plasma was extracted with hexane for analysis of carotenoids by HPLC with electrochemical detection [24]. Fatty acids were analyzed in plasma phospholipids using Folch extraction of plasma and phospholipid isolation using sep-paks [25]. Derivatization was with Methanolic KOH, using dry conditions, which esterifies all classes of fatty acids well [26]. Individual fatty acids were quantified by gas chromatography with mass spectral detection using selected ion monitoring of either a most abundant ion or most characteristic ion for each fatty acid. Heptapentanoic acid was the internal standard. Cholesterol was determined with enzymatic assay using the Infinity Cholesterol Reagent from Thermo Electron (Louisville, CO).

NAF was assayed for cholesterol by the same method as for plasma, using 5 μ l of the diluted NAF. Protein in NAF was assayed with Advanced ADV01 reagent from Cytoskeleton (Denver, CO). C-reactive protein in both NAF and plasma was assayed using a high sensitivity ELISA kit, IB6100, from IBL-America (Minneapolis, MN). C-reactive protein and cholesterol levels in NAF were expressed per microgram of protein in NAF.

Epithelial cells were counted on each slide, but when there were large numbers of cells or clumps of cells present, this was estimated using a grid to count only a portion of the slide. The morphometric measurements on epithelial nuclei were made using an Olympus BX-40 microscope and MicroComputer Imaging Device (MCID) software model M5+ (Imaging Research Inc., Brock University, St. Catharines, Ontario) with a 2-monitor system in 1280 $\times$ 1600 pixel format. The measures were made with the basic morphometric software provided by MCID. Nuclei to be imaged were identified manually but delineated automatically by the software. The measures were performed on a minimum of 50 epithelial cells/slide whenever possible. Three slides with only one to three well-delineated nuclei were excluded from statistical analyses. The average number of nuclei measured was 51 per slide (median 52 per slide) and the range was 7 - 105 cells.

The nuclear features captured in this study were: nuclear area, perimeter, clipped perimeter, maximum straight cord, mean diameter, maximum diameter, circular diameter, length, form factor (roundness), major axis length and minor axis length. All the measures were taken by a single observer, but atypia was confirmed by a 2nd clinical cytopathologist. Measures of nuclear density were not as reproducible being dependent on staining, clumping of cells and microscope light level, and therefore were not analyzed in this study. Three slides were each imaged on three separate days to assess intra-observer variability. The coefficient of variation (CV) for the three days of measurements ranged from 6% to 14% with a mean of 9%. The CV for form factor was 7% and for nuclear area it was 14%. This is much smaller

than the variation among cells on a single slide with a mean CV of 22%, range 15-34% for the individual measures (15% for form factor and 34% for nuclear area).

2.4 Statistical methods

All statistical analyses were performed with Graph-Pad Prism 5 software for Windows (San Diego, CA). For two-group comparisons of continuous variables, the two-sample t-test or the Mann-Whitney test was used. For categorical variables, Fisher's Exact test was used. Spearman correlations were used to explore associations among 20 dietary, demographic and breast variables: total plasma carotenoids, plasma fatty acids (percentage MUFA, n-6 PUFA, SFA), plasma cholesterol, plasma C-reactive protein, NAF C-reactive protein, total dietary carotenoids, Healthy Eating Index, BMI, age, exercise, 5-year Gail risk score and morphological features of the epithelial cells (form factor, form factor CV, diameter, perimeter, major axis length, minor axis length, maximum chord). Alpha of 0.05 was adjusted by the method of Holm to avoid type I errors when making multiple comparisons [27].

3. Results

There were 57 women enrolled in the study, and their demographic characteristics are shown in Table 1. About half of the women were African American. The clinical diagnoses of enrolled women were: sclerosing adenosis (n = 19), hyperplasia (designated as either moderate, florid or proliferative, n = 27, including one with radial scar), and papilloma (n = 11). The biopsies had been done 26 years to 2 weeks (median 6 years) before enrolling in this study. The ductal lavage procedure was attempted on the breast ipsilateral to the previous biopsy first, but if this was not fruitful the procedure was attempted on the contralateral breast. The contralateral breast was lavaged in 5 of 36 cases.

Gail risk score was calculated for the women using the Health Status Questionnaire data. The 5-year risk estimates ranged from 0.4 to 5.3%, with no scores calculated on the two women who were under 35 years of age. Thirteen of the 57 women had Gail risk scores above 1.66%, which would classify them as being at high risk for breast cancer. Scores by ability to yield NAF are shown in Table 2, and were slightly higher in women who did not produce NAF.

Intakes of nutrients were assessed using the Block '98 Food Frequency Questionnaire and there were 55 women with valid data. Mean fruit and vegetable intakes were 4.5 servings/day, which is close to the mean intake for Americans but below current recommendations. Fat intake was somewhat higher than the average American intake at 38% of energy from fat (SD 7, n=55). The mean Healthy Eating Index (HEI) score of 60 (SD 12) indicated that the subjects' diets, on average, need improvement [28].

Of the 57 women enrolled, 39 yielded nipple aspirate fluid (NAF) samples for a success rate of 68%. It should be noted, however, that these data are from a single attempt at NAF production and yields of NAF might have been better with repeat visits. The volume of NAF collected was very small, about 5 μ l, and was not measured in most cases. Women who yielded NAF were of significantly younger age than women who did not yield NAF (Table 2). Production of NAF was not significantly associated with BMI, smoking, HEI score, Gail risk score or race (Table 2). There were also no differences in any of the plasma blood measures with ability to produce NAF, $p > 0.1$ in each case (data not shown). Levels of NAF C-reactive protein were higher in African American women with a mean of 1.68 (SD 1.69) than in European American women with a mean of 0.73 (SD 0.77), given in μ g/ μ g protein ($p = 0.027$ from two-sample t-test). Levels of NAF cholesterol were similar by race.

Eight women who provided NAF did not undergo successful lavage because of failure to seat the catheter in the duct; conversely, 82% of women who produced NAF underwent lavage. Five women who did not yield a NAF sample did undergo successful lavage, leaving a total of 36 women with successful breast ductal lavage (63% of those enrolled). Of the 36 women with successful lavage, five had samples that were acellular, three had 30 cells or less and the rest (28, or 77% of all lavages) had 200 to more than 10,000 cells. Increased cellularity in NAF has been shown to be a risk factor for breast cancer in prospective studies [29,30], but has unknown significance in ductal lavage samples. A total of 11 women had atypia in the epithelial cells, and these 11 samples all had more than 200 cells total. Two of the samples had marked atypia. There were 13 women with 5-year Gail risk scores of 1.7% and higher, and the ductal lavage success rate was similar in that sub-group with 6, or 46%, completing lavage procedure and all 6 having 200 cells or more.

The characteristics of women who provided lavage samples, given by whether or not atypia was present, are given in Table 3. Women with epithelial atypia had a higher mean BMI than women without atypia in their lavage samples. All the women with atypia were either overweight or obese. Women with atypia also reported using aspirin somewhat more often indicating the possibility of an underlying inflammatory condition. Cellularity in the samples without atypia was also associated with BMI. For the 25 women with >200 cells, 65% were overweight or obese and for the 8 women with 30 cells or less, only 38% were overweight or obese. There were no significant differences in the other variables by presence of atypia including plasma measures, NAF measures, diet and exercise. There were no statistically significant relationships of atypia to the nuclear measures as well, but there was a suggestion that form factor and variability (coefficient of variation) in the form factor was higher in women with atypia. All the area and length-related morphological features had higher means in women with atypia but the differences were small and not statistically significant. Figure 1 shows the morphometric features most strongly associated with atypia.

BMI was strongly and significantly correlated with NAF C-reactive protein ($r = 0.558$, $p < 0.001$) and plasma C-reactive protein ($r = 0.390$, $p = 0.002$), but BMI was not significantly correlated with NAF or plasma cholesterol. A few other variables of interest were correlated with BMI but they did not reach significance after adjusting alpha for the 20 multiple comparisons: exercise (in hrs/week) ($r = -0.385$), n-6 PUFA levels in plasma ($r = 0.357$), and blood carotenoid levels ($r = -0.368$).

Of the morphological nuclear features of cells from breast lavage, none of the correlations with BMI were statistically significant, with the CV of form factor having the strongest non-significant correlation of $r = 0.365$, $p = 0.057$. Form factor of nuclei, which was available from the 28 women with seven or more distinct cells imaged, was not correlated significantly with any of the other variables examined. The CV for form factor decreased as mean form factor increased ($r = -0.919$, $p < 0.0001$), and the standard deviation of form factor also decreased as mean form factor increased ($r = -0.878$, $p < 0.0001$). Since a form factor with the maximal value of 1.0 would be a perfect circle, this indicates that samples with lower mean form factor values exhibited relatively more variation in nuclear shape, as indicated by higher standard deviation and CV.

Associations of NAF C-reactive protein with the other measures also were explored using Spearman correlations. Since NAF volume was difficult to measure, levels of NAF C-reactive protein were normalized per amount of NAF protein. The volumes of NAF were small in all cases (1-10 μ l), and correlations of C-reactive protein per volume of diluted NAF or per mg protein gave very similar results (data not shown). C-reactive protein levels in plasma and NAF were not well correlated with each other ($r = 0.287$, $p = 0.076$). The only significant correlation of NAF C-reactive protein was with BMI, as shown in Figure 2. The

correlation was somewhat stronger for African American women (Spearman $r = 0.598$) than for European American women (Spearman $r = 0.415$). None of the other variables were significantly correlated with NAF C-reactive protein.

4. Discussion

Breast ductal lavage was developed as a means to sample or monitor the breast ductal epithelium. The present study of women with a past diagnosis of proliferative breast disease indicates that lavage is unlikely to be useful for biomarker endpoints in more than half the of the study sample. This is similar to that of previously published studies of ductal lavage, with epithelial cells being present in 52 to 88% of lavages from high risk women and in 56% of lavage samples from average risk women [31-37].

One somewhat surprising finding was the presence of epithelial atypia in almost one third of those women lavaged. Our study was small, but there are other reports of atypia in a substantial portion of women who undergo breast ductal lavage. Atypia in breast ductal lavage in an Italian study was found to be frequent in women at increased risk for breast cancer: 52% in women with previous contralateral breast cancer and 22% in normal risk women [37]. The rate of atypia was 21% in women with BRCA1 or 2 mutations [36]. It should be noted, however, that the significance of atypia in ductal lavage is unclear. The sloughing of atypical cells may be a protective mechanism. Repeat ductal lavage of women at a 6-month interval has indicated that presence of benign or atypical cells in lavage is not reproducible in women at increased risk for breast cancer [35], and cytological readings between lavage and random fine needle aspiration or subsequent biopsy were only concordant 52-63% of the time [32,33]. In addition, inter- and intra-observer agreement has been found to be low to moderate for cytological readings [38], and a limitation of our study is that we had only one observer. Finally, while the relationship of atypia in ductal lavage and subsequent breast cancer risk is not yet established, presence of atypia in nipple aspirate fluid increased the relative risk for breast cancer risk to 2.0 (versus women who did not produce fluid), indicating that atypia is not a particularly strong risk factor [29]

Interestingly, BMI was strongly related to presence of atypia, and all the women with atypia were either overweight or obese. In a study using random fine needle aspiration of women at high risk for breast cancer, Masood cytology index score of the breast epithelial cells also was significantly higher in women who were overweight or obese [39]. The association of BMI with atypia has implications for design of preventive strategies centered on weight control. Adult weight gain and obesity after menopause have been associated with increased breast cancer risk [40,41]. Interestingly atypia was not associated with other factors, like Gail risk score. Bushnaq et al. also found that Gail risk did not predict atypical cytology in ductal lavage [42]. However, C-reactive protein in NAF was significantly associated with BMI in the present study, more strongly so than with plasma C-reactive protein, and may indicate a pro-inflammatory state that can facilitate cell transformation. This could be mediated by obesity-associated adipokines and hormones, such as leptin and insulin [43]. The association of BMI with C-reactive protein has also been found in one other study of a heterogeneous population of women that included both African and African American women [44,45].

One important goal of the present study was to examine the morphology of the cells obtained by lavage in relation to nutritional factors. Morphology by image analysis could be useful to quantify subtle changes in a chemoprevention setting [46]. This would then provide a continuum of changes to examine in a prevention setting that goes beyond the traditional pathological categories of benign, atypia and malignant. For example, in a study of random fine needle aspiration of the breast in high risk women, letrozole use affected

nuclear area, optical density and non-uniformity of staining in the nuclei [47]. In two prospective studies of women with benign breast disease, nuclear shape has been shown to be predictive of subsequent breast cancer risk [20,48].

There are many nuclear features which can be quantified, and some have emerged to be more useful than others. In a study of ductal carcinoma in situ with image analysis of surgical specimens, Axelrod et al found that the ellipticity of nuclei was related to recurrence rates [49]. Although relatively more studies have focused on analysis of nuclear features as a prognostic variable for cancer, there are studies of morphological changes early in the transformation process. Nuclear area of epithelial cells obtained by FNA was altered in women with hyperplasia versus with normal epithelium [50]. Nuclear features of moderate hyperplasia and atypical hyperplasia were not distinguishable and were intermediate between carcinoma and non-proliferative tissue [51]. In another study, nuclear area variation, cell size, and large nucleoli were the most robust features for cytological characterization of ductal lavage fluid [52]. The present study was small, but form factor and variability in form factor were the nuclear features more strongly associated with presence of atypia. The other nuclear features that contribute to form factor, eg. area, diameter, perimeter, length, were all slightly higher in samples with atypia (Figure 1).

In summary, the major finding of this study was the association of overweight and obesity with epithelial atypia in breast ductal samples from women with a past diagnosis of proliferative breast disease (Figure 2). There was little evidence of a role for dietary factors in mediating this association. There was, however, an indication that this may be related to a pro-inflammatory state induced by increased body weight since C-reactive protein levels in NAF were closely correlated with obesity. Such data support the further investigation of the role of overweight and obesity in modulating breast cancer risk.

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Figure 1.

Morphometric features of epithelial cells in ductal lavage samples with and without atypia. Samples with a minimum of 10 imaged cells are shown: there were 18 without atypia and 10 samples with atypia. The p-values shown are for t-tests except for the CV of form factor and maximum chord length for which the Mann-Whitney test was used.

Figure 2. C-reactive protein levels in nipple aspirate fluid (NAF, in ng/mg total protein) by BMI. The Spearman correlation coefficient was $r = 0.558$ ($p=0.0003$).

Table 1

Demographic characteristics of the 57 women enrolled in the Benign Breast Markers Study.

Characteristic	Mean, SD or number (percent of total)
Age at enrollment	51, 9
BMI (kg/m ²)	29.3, 7.5
Race ^a	28 (49%) European American 29 (51%) African American
Married	28 (49%)
College Graduate	29 (51%)
Employed	40 (70%)
Current Smoker	12 (21%)
Parous	45 (79%)
Lactated	19 (33%)
Post-menopausal ^b	38 (67%)
Age at menarche, years	12.7, 2.0
Family history of breast cancer ^c	8 (14%)
Family history of other cancer ^c	16 (28%)
Gail risk score (5 year risk, %) ^d	1.48, 0.75

^aOne African American woman was of mixed Hispanic ethnicity.

^bWomen were classified as post-menopausal if it was more than three months since their last period. Only one woman was using oral contraceptives.

^cHistory of breast or other cancers in primary relatives.

^dGail risk score was obtained for 55 women with a web-based breast cancer risk assessment tool [23]. The score could not be calculated on two women who were under the age of 35.

Table 2

Characteristics of subjects who did and did not produce nipple aspirate fluid (NAF). Table entries are mean and SD, or number and percentage.

Characteristic	no NAF n=18	produced NAF n=39	P-Value ^a
Age	56, 9	49, 8	0.003
Postmenopausal	16 (88%)	22 (56%)	0.018
BMI	29.0, 7.8	29.4, 7.5	0.925
Current Smoker	2 (11%)	10 (26%)	0.303
Gail risk score, 5-year % ^b	1.77, 1.12	1.32, 0.42	0.122
African American	6 (33%)	23 (59%)	0.092
Parous	16 (89%)	29 (74%)	0.303
Lactated	4 (22%)	15 (38%)	0.365
Healthy Eating Index score	59.4, 12.7	60.2, 12.0	0.891

^aThe p-values shown are from the Mann Whitney test or Fisher's Exact test for proportions. None of the differences, except for age, were statistically significant after adjustment of alpha for 9 multiple comparisons by the method of Holm [27].

^bTwo women under the age of 35 on whom Gail risk score could not be calculated did not produce NAF.

Table 3

Characteristics of subjects by presence or absence of atypia in epithelial cells from breast ductal lavage. Women with acellular ductal lavage samples are included in the “no atypia” category. Table entries are mean and SD, or number and percentage, and the p-values are from the Mann-Whitney test or Fisher’s Exact test for proportions.

Characteristic	No atypia n = 25	Atypia ^a n = 11	P-Value
Age, years	51, 8	49, 7	0.354
Smoker	8 (32%)	2 (18%)	0.688
Recent NSAID use ^b	15 (60%)	9 (82%)	0.268
BMI, kg/m ²	27.4, 6.9	35.1, 7.7	0.008 ^c
Obese	7 (28%)	7 (64%)	0.067
Gail risk score	1.32, 0.42	1.29, 0.59	0.430
African American	14 (56%)	7 (64%)	0.729
Took aspirin in the past 2 weeks	15 (60%)	9 (82%)	0.268
Plasma C-reactive protein, µg/ml	1.20, 0.96	2.84, 3.57	0.164

^a All slides with atypia had more than 250 epithelial cells present.

^b The Health Status Questionnaire asked about aspirin and other NSAID use in the past two weeks.

^c Significantly different after adjusting alpha for nine simultaneous comparisons.