Association of Age and Reproductive Factors with Benign Breast Tissue Composition[1]

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Abstract

Reproductive breast cancer risk factors are hypothesized to act by increasing exposure of the breast to endogenous estrogens, but few studies have quantitatively examined the association of these risk factors with breast tissue composition. This study is part of a case-control study of breast histological characteristics and breast cancer risk, nested within the Nurses' Health Study, a prospective study of 121,700 registered nurses. We studied 300 women who had not been diagnosed with breast cancer, but for whom we obtained slides from a prior benign breast biopsy. We used a computer-assisted image analysis technique to assess the proportion of epithelial and fibrous stromal tissue on benign breast biopsy slides, excluding obvious mass lesions. Mean epithelial proportion was 5.3% (0.1–23%), and mean stromal proportion was 58.7% (3–93%). Women with proliferative breast disease without atypia had higher epithelial and stromal proportions than women with nonproliferative breast disease ($P < 0.001$). Postmenopausal women had a lower epithelial proportion ($P = 0.01$), and increasing age at biopsy was associated with decreasing stromal proportion among postmenopausal parous women ($P = 0.004$). Among premenopausal women, increasing years since last birth was associated with lower epithelial proportion ($P < 0.001$). Other reproductive risk factors were not independently associated with epithelial or stromal proportion. Epithelial and stromal breast tissue were associated with different factors with the exception of proliferative breast disease, which was associated with an increase in both epithelial and stromal proportion. The quantitative measurement of epithelial and stromal proportion may be useful for measuring changes in breast composition.

Introduction

Recognized breast cancer risk factors, such as early age at menarche, older age at first birth, and late age at menopause, are hypothesized to act via increasing cyclic exposure to estrogen and progesterone, resulting in proliferation of breast epithelial cells (1, 2). Relatively little is known about the effect of reproductive risk factors on breast tissue composition, which may be an important factor in understanding the mechanism of breast cancer development. The composition of breast tissue may be a marker of cumulative effects of endogenous hormones and may represent an intermediate marker of breast cancer risk.

The breast is composed primarily of supporting connective tissue (stroma) and adipose tissue. The density of the breast is related to the relative proportions of stroma, adipose tissue, and epithelium, which lines the ductal system (3). Substantial changes occur in breast composition in response to hormonal variations as the breast undergoes differentiation during pregnancy and subsequent involution after menopause (4, 5). Breast involution following menopause is associated with regression of lobules and a relative increase in adipose tissue and stroma (6). Lobule development increases dramatically during pregnancy, persists during lactation, and subsequently undergoes postlactational involution. Numerous hormones affect breast epithelial tissue: development and growth of the ductal system are influenced by estrogen, and progesterone in the presence of growth factors promotes development of lobules (6–8). Stromal proliferation and involution are thought to be determined locally by a complex balance between degrading proteinases and their inhibitors. In addition, stromal factors have been shown to be involved in the regulation of epithelial proliferation (9, 10).

Our aim in this study was to quantitatively determine the association of age and reproductive factors on breast tissue composition, using a novel application of a computer-assisted image analysis technique to measure the proportion of epithelium and fibrous stroma of breast tissue from benign breast biopsies.

Materials and Methods

Study Design. The study was conducted within a case-control study of breast histological characteristics and breast cancer risk nested in the Nurses' Health Study, an ongoing prospective study of 121,700 nurses 30–55 years of age at baseline in 1976. The case-control study has been described in detail (11, 12). Women who reported a history of prior breast biopsy on their biennial questionnaires were eligible for this study. Breast

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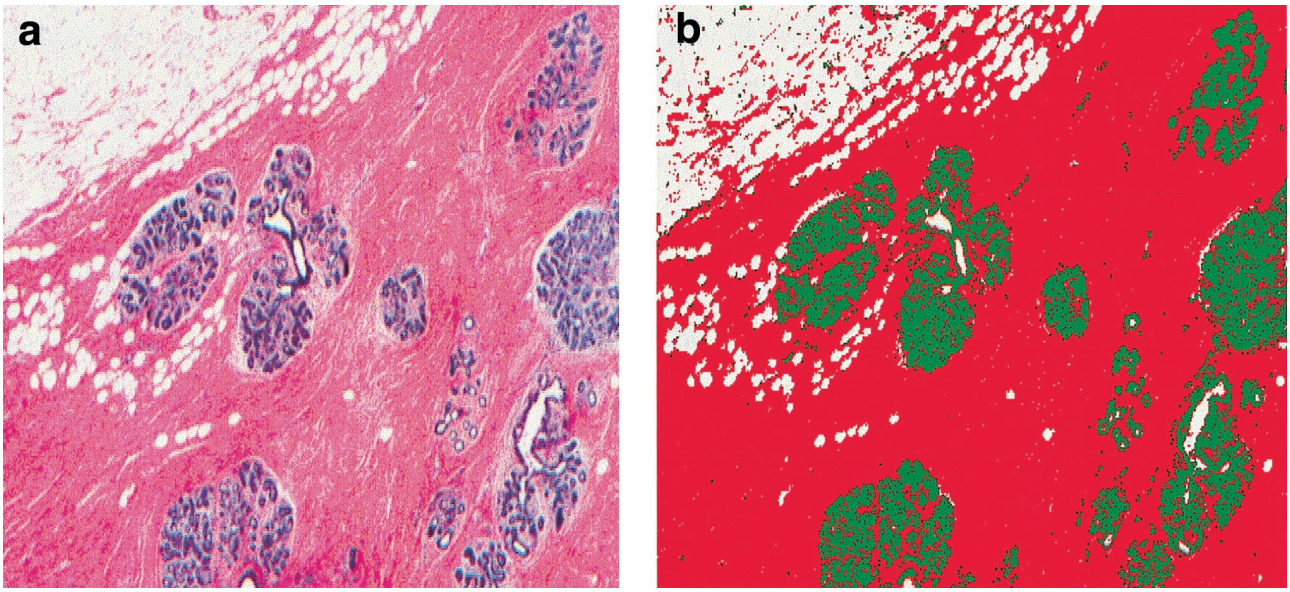

Fig. 1. *a*, example of an area of a scanned breast biopsy slide (H&E stain). *b*, the same area of the breast tissue image with thresholds set for epithelial tissue (*green*) and stromal tissue (*red*), using computer-assisted image analysis.

cancer cases diagnosed between 1976 and 1990 were determined by self-report of breast cancer on questionnaires sent every 2 years and confirmed by medical record review. In the original study, four controls who had also reported a breast biopsy but were free from breast cancer at the time of case diagnosis were matched to each case according to age at breast cancer diagnosis and year of breast biopsy. With permission from the study participants, slides from the earliest available breast biopsies were sought from the hospitals where the biopsies were performed.

All controls with available slides from the 1988–1990 follow-up cycles of the Nurses' Health Study were included in the present study ($n = 300$). A number of sets of slides from the original study, predominantly from the 1976–1986 follow-up, were returned to the hospitals during the course of the study ($n = 488$ controls); thus, for the purpose of this study, controls from these follow-up cycles are not included to avoid potential for bias. Further exclusions are described below.

Measurement of Exposure. We developed a novel application of a computer-assisted image analysis method for quantitatively measuring the proportion of epithelium and stroma of breast tissue on breast biopsy histology slides. Two slides from the breast biopsy (or two different pieces of tissue on the same slide if only one slide was available) were selected at random for each subject and reviewed in a blinded fashion by a pathologist (I. S.) for suitability for analysis. Slides were excluded if they were of poor technical quality ($n = 5$ women), if lactational changes were present ($n = 2$ women), or if an obvious mass lesion such as fibroadenoma was present with no surrounding normal tissue ($n = 20$ women) because these conditions would affect the measurements. Slides that were very faded or poorly stained were restained with H&E ($n = 170$ slides). The slides were then scanned with a Sprintscanner (Polaroid, Cambridge, MA). Combinations of color and intensity thresholds for epithelial and stromal tissue were set manually with the software program Sigmascan Pro 4.0 (SPPS, Chicago, IL). The outer boundary of the scanned tissue image was first traced manually to calculate the total tissue area, and the area external to that was then eliminated with the masking feature of the software. The color threshold for epithelium was adjusted visually, using the intensity feature of the software for each image as necessary to include all of the epithelial area. For the stromal measurement, a different manual intensity threshold was set that also included the epithelial area; the cross-sectional area occupied by the epithelial and stromal thresholds was then calculated. The epithelial area was subtracted from the stromal area in the calculations of stromal proportion. Lastly, the percentage of the total tissue area on the breast biopsy slides occupied by epithelium and stroma was calculated by dividing the epithelial and stromal measurements by the total tissue area. For all analyses, the average measurement of the two slides was used as the outcome variable. In this report, we refer to the average epithelial proportion of the total breast tissue area on the scanned breast biopsy slides as the epithelial proportion and the average stromal proportion of total breast tissue area on the scanned biopsy slides as the stromal proportion. Examples of thresholds for epithelium and stroma are shown in Fig. 1. The majority of thresholds were measured by a trained research assistant, with difficult images referred to either D. G. or I. S.

Reproducibility Study. Thirty-eight slides were selected at random from the scanned images for the reproducibility study. To assess reproducibility of the measurement, two measurements on each slide were performed independently by the research assistant, separated by at least 1 week. In addition, three observers (D. G., I. S., and the research assistant) independently and blindly measured epithelial and stromal thresholds to evaluate the interobserver reproducibility. Reproducibility of the measurement by the research assistant, who was the primary reviewer and performed the measurements on all subjects, was high; the Spearman correlation coefficient was $r = 0.92$ for both epithelium and stroma. For the interobserver reproducibility, Spearman correlations for the epithelial measurements were as follows: $r = 0.89$ between I. S. and D. G.; $r = 0.81$ between I. S. and the research assistant; $r = 0.84$ between D. G. and the research assistant, ($P < 0.001$). For the stromal measurements, correlations were $r = 0.77$ between I. S. and

Table 1 Age-adjusted means of epithelial and stromal proportion, by breast cancer risk factors, for all women and for parous women only[a]

	N[b]	Epithelium			Stroma		
		Mean (%)[c]	SE	*P*	Mean (%)[c]	SE	*P*
All women							
Age at biopsy (years)							
<30	28	7.5	0.7	<0.001[d]	67.9	3.6	<0.001[d]
31–40	57	6.0	0.5		63.1	2.5	
41–50	118	5.5	0.3		61.1	1.7	
51–60	76	4.2	0.4		53.4	2.2	
>60	21	3.1	0.8		40.2	4.1	
Menopausal status							
Premenopausal	194	5.8	0.3	0.02	60.9	1.4	0.05
Postmenopausal	98	4.4	0.4		54.8	2.4	
Age at menarche (years)							
<12	59	5.1	0.4	0.44[d]	57.0	2.5	0.04[d]
12	73	5.0	0.4		56.1	2.5	
13	95	5.5	0.4		59.1	1.9	
≥14	70	5.4	0.4		62.5	1.9	
Family history of breast cancer							
No	257	5.2	0.2	0.22	58.4	1.2	0.57
Yes	43	6.0	0.5		60.2	2.7	
BMI (quartile)							
1	75	5.6	0.5	0.51[d]	61.1	1.9	0.01[d]
2	73	5.2	0.4		58.1	2.2	
3	78	5.4	0.4		60.4	2.1	
4	74	4.9	0.4		55.1	2.4	
Height in 1976 (inches)							
<62	66	5.5	0.5	0.50[d]	61.7	2.2	0.14[d]
62–65	106	5.4	0.3		56.9	1.8	
≥66	128	5.1	0.3		58.7	1.7	
Benign breast disease							
Nonproliferative	109	4.2	0.3	<0.001	55.2	2.1	0.04
Proliferative without atypia	151	6.1	0.3		61.3	1.3	
Atypical hyperplasia	40	5.1	0.5		58.3	3.2	
Oral contraceptive use							
Never	109	5.3	0.3	0.21	60.6	1.4	0.003
Ever	169	4.8	0.3		53.7	1.8	
Parity							
Nulliparous	32	4.0	0.6	0.04	66.4	2.4	0.02
Parous	257	5.4	0.2		57.8	1.2	
Parous women							
Parity							
1–2	104	4.6	0.3	0.05[d]	57.5	1.9	0.66[d]
3–4	121	5.8	0.3		56.9	1.7	
>4	32	5.5	0.6		58.0	3.4	
Age at first birth (years)							
0–24	133	4.9	0.3	0.44[d]	57.2	1.7	0.46[d]
25–29	91	5.6	0.4		56.1	2.0	
≥30	17	6.0	0.8		63.5	4.6	
Time since last birth (years)							
1–4	18	8.8	1.4	<0.001[d]	49.7	6.0	0.04[d]
5–9	47	6.8	0.5		62.0	3.2	
10–14	52	4.9	0.5		61.3	2.8	
15–19	44	4.9	0.5		55.4	2.9	
≥20	83	3.5	0.5		52.3	3.0	

[a] All variables at time of biopsy unless otherwise indicated.
[b] Numbers do not all add up to 300 because of missing data. Two women are missing data for the epithelial measurement.
[c] Age-adjusted mean.
[d] *P* for test for trend for continuous variables.

D. G.; $r = 0.72$ between I. S. and the research assistant; and $r = 0.92$ between D. G. and the research assistant ($P < 0.001$). These correlations are comparable to those obtained in mammographic studies using computer-assisted techniques to measure breast density (13).

Measurement of Covariates. All breast biopsy slides were centrally reviewed by pathologists at the Beth Israel Deaconess Medical Center (S. S. and J. C.), and a standard classification for benign breast disease, based on the Page classification, was used (14). For all women, data on the following variables were obtained by questionnaire: age at menarche, age at menopause, parity at diagnosis, history of breast feeding, family history of breast cancer, and weight and height in 1976. Parity at biopsy, time since last birth, and age at first birth were calculated using

the year of the breast biopsy, and the age of the children in 1978 (where this information was missing, the year of birth of any children from the 1996 questionnaire was used). In calculations of time since last birth, we excluded women who gave birth in the same year as their reported year of biopsy because we were unable to determine the month of birth. Oral contraceptive use at the time of biopsy was calculated using the intervals of ever use reported on the 1976 questionnaire for women biopsied before 1976 and from the biennial questions on oral contraceptive use if they were biopsied after 1976. Menopausal status at biopsy was determined by subtracting the age at menopause as reported in 1992 from the woman's age at biopsy. Women who had had surgery with at least one ovary remaining or whose type of menopause was unknown were excluded from the estimates of menopausal status.

Statistical Analysis. To assess the associations of reproductive risk factors with epithelial and stromal proportion, we first performed bivariate analyses, adjusting for age at biopsy as a continuous variable with linear regression models to predict epithelial and stromal proportion. Reproductive variables such as age at first birth, time since last birth, and breast feeding were assessed among parous women. We then used multivariate linear regression to determine independent predictors of epithelial and stromal density and developed a parsimonious model that best described the data, using the significance of variables at $P < 0.10$ and *a priori* information about the predictive value of the variables (15). In addition, the results of the reduced model were compared with those from a full model, which included all of the covariates of interest. Non-reproductive risk factors were assessed among all women, with the adjustment for reproductive risk factors modeled as indicator terms from a categorical variable combining parity (1 or 2, >2), age at first birth (<25, 25–29, ≥30 years), and time since last birth (continuous), with nulliparous women as the reference group. Averaged epithelial and stromal measurements were analyzed on the natural scale. To account for the heteroscedasticity and nonnormality of regression residuals, we used the robust variances for Wald-type inferences to obtain *P* values and confidence intervals (16). There were no substantial differences between the *P* values calculated in this manner and the empirical *P* values. Two outliers were excluded from calculations of epithelial proportion after re-evaluation of their original images showed them to have lactational changes.

Results

In this study, the mean epithelial proportion was 5.3% (SD, 3.7%; range, 0.07–22.5%) and the mean stromal proportion was 58.7%, (SD, 19.9%; range, 3.0–93.3%). The mean age at biopsy was 45.2 years (range, 20–66 years), and the year of biopsy ranged from 1950 to 1990, with a median of 1978. The correlation between the two epithelial measurements for each woman was 0.51, $P < 0.001$, and between the stromal measurements was 0.70, $P < 0.001$. The epithelial and stromal measurements were also positively correlated, $r = 0.31$, $P < 0.001$.

In the univariate analyses, epithelial proportion was lower among older women at biopsy (Table 1). In the age-adjusted analyses, epithelial proportion was lower for postmenopausal women, $P = 0.02$. Women with proliferative breast disease without atypia had higher epithelial proportion (6.1%) than women with nonproliferative breast disease (4.2%; $P < 0.001$); women with atypical hyperplasia had an intermediate proportion of epithelial tissue. Other known breast cancer risk factors, in particular family history, age at menarche, and age at first birth, were not significantly associated with epithelial proportion. As shown in Fig. 2, increasing time since last birth was associated with a decline in epithelial proportion among parous women. In multivariate linear regression models that examined the associations of non-reproductive risk factors, the difference between the adjusted mean epithelial proportion in pre- and postmenopausal women was $\beta = -1.56\%$, $P = 0.01$; the presence of proliferative breast disease without atypia and the presence of atypical hyperplasia were significant positive predictors of epithelial proportion (Table 2). In multivariate analyses among parous women, increasing time since last birth was associated with a significant decline in epithelial proportion per year, and this effect was stronger among premenopausal women ($\beta = -0.26$, $P < 0.001$). There were no significant associations between epithelial proportion and history of breast-feeding, parity, or age at first birth. Approximately 20% of the variability in epithelial proportion was explained by the covariates included in the models.

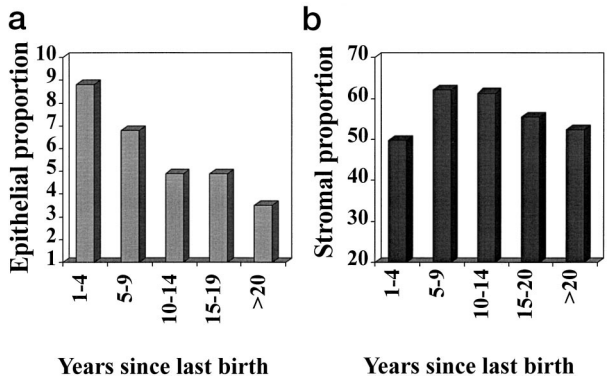

Fig. 2. Age-adjusted means of epithelial (*a*) and stromal (*b*) proportion by time since last birth among parous women only.

Stromal proportion was also inversely related to age at biopsy (Table 1), although this decline was greater after 50 years of age. Being parous and ever having used oral contraceptives were associated with a lower stromal proportion in age-adjusted analyses. Conversely, stromal proportion was greater among women with proliferative disease without atypia. Table 3 shows the results from the multivariate linear regression for stromal proportion. Although there was no overall effect of age at biopsy, we observed a significant negative interaction between age at biopsy and menopausal status among parous women (not shown in Table 3). After inclusion of the interaction term, stromal proportion declined by −1.53% per year with increasing age among parous postmenopausal women ($P = 0.004$). Ever having used oral contraceptives remained inversely associated with stromal proportion, although this was limited to premenopausal women in stratified analyses (−8.6% difference between ever and never users, $P = 0.002$). However, an interaction term between oral contraceptive use and menopausal status was not significant in the overall model. Among parous women, no association was seen between stromal proportion and time since last birth or other parity-associated variables after controlling for age at biopsy (Table 3).

We performed analyses stratified by menopausal status to

Table 2 Predictors of epithelial proportion among all women, controlling for variables relating to parity, and among parous women only

A. All women	Full model[a] $r^2 = 0.21$		Parsimonious model[a] $r^2 = 0.20$	
Predictors	β	P	β	P
Age at biopsy (years)	−0.04	0.32	−0.03	0.41
Menopausal (yes/no)[b]	−1.58	0.01	−1.56	0.01
Proliferative without atypia (yes/no)	1.81	<0.001	1.80	<0.001
Atypical hyperplasia (yes/no)	1.29	0.03	1.38	0.02
Oral contraceptive use (ever/never)	−0.43	0.30		
Family history (yes/no)	0.72	0.25		
Age at menarche (years)	0.09	0.63		
BMI (weight/height^2)	−0.003	0.96		
Height in 1976 (inches)	−0.06	0.48		
B. Parous women only	**Full model[c] $r^2 = 0.29$**		**Parsimonious model $r^2 = 0.24$**	
Predictors	β	P	β	P
Time since last birth (years)				
Premenopausal women	−0.31	<0.001	−0.26	<0.001
Postmenopausal women	−0.09	0.20	−0.08	0.13
Age at first birth (years)	−0.01	0.80		
Parity (0–9)	−0.06	0.76		
History of breast feeding (yes/no)	0.50	0.29		

[a] Controlling for cross-classified term including parity (0, 1–2, >2), age at first birth (≤24, 25–29, >30 years), and time since last birth in single years and a missing indicator term.
[b] From a model with missing menopausal status excluded, controlling for other covariates.
[c] Also controlling for menopausal status (pre-, post-, missing), interaction between menopausal status and time since last birth (continuous), categories of benign breast disease (nonproliferative, proliferative, and atypical hyperplasia), family history (yes/no), age at biopsy (years), oral contraceptive use (ever, never, missing), BMI (weight/height^2), and height (inches).

examine potential differences in influence of BMI[3] for pre- and postmenopausal women. In age-adjusted analyses among premenopausal women, there was a modest nonsignificant decline in epithelial proportion with increasing BMI that was not present in multivariate analyses. The influence of BMI on stromal proportion did not differ for pre- or postmenopausal women (data not shown). When we stratified by presence of nonproliferative *versus* proliferative benign breast disease, the risk factors were in the same direction for both strata of benign breast disease for epithelium and stroma, although less significant because of smaller numbers in each stratum. The only exception was family history, which was non-significantly inversely associated with stromal proportion in the proliferative stratum, $\beta = -2.68$, $P = 0.45$, and was positively associated in the nonproliferative stratum, $\beta = 5.76$, $P = 0.38$.

We had information on postmenopausal hormone use for 73 women who had biopsies after 1976. With control for age at biopsy and age at menopause, the epithelial proportion for current hormone users was 3.7% and for never users was 3.1%, $P = 0.35$. Current users had a nonsignificantly higher stromal proportion, 51.7%, than never users, 43.1%, $P = 0.15$.

Discussion

Our results show that age, menopausal status, and time since last birth are significantly associated with benign breast composition, with differences for epithelial and stromal proportion. These findings are consistent with other studies that have examined the effects of age and parity on the breast; however, in our study we were able to control simultaneously for a variety of reproductive factors and used a continuous measurement of breast composition based on a computer-assisted image analysis technique.

Much of the work on breast tissue composition during the reproductive life span has been done by Russo *et al.* (4, 17–19), who have described three major types of breast lobules ranging from type 1, the least developed, to type 3, the most complex branching type with most epithelial tissue. The authors have shown that parous women have a greater proportion of type 3 lobules than nulliparous women, which regress to predominantly type 1 lobules after menopause, when they are histologically indistinguishable from those of nulliparous women but are much more sensitive to carcinogens (5, 18). Thus, our observation that the proportion of epithelial tissue in the breast regresses with menopause and increasing time since last birth, even after adjustment for age, is consistent with the findings of Russo *et al.* (5, 18). We did not examine lobule type in this study; however, we would expect that the epithelial proportion would increase with more complex branching lobule types, which by definition contain greater amounts of epithelium.

Few other studies have quantitatively assessed breast tissue composition, although changes in appearance of breast tissue with age, menopause, and parity are widely accepted. Declines in both epithelium and stroma with age were reported in a study using morphometric techniques, but the authors did not observe a relationship with parity, possibly due to the small numbers of subjects (20). In another study, lobule development in mastectomy specimens was classified into four categories, ranging from "none" to "good," and a decline in lobular development in postmenopausal women was observed (21).

The estimate of breast tissue age used in models of breast cancer incidence is postulated to reflect an accumulation of genetic damage, which is modified by the terminal differentiation of the breast with first and subsequent pregnancies (22–24). These models predict a short-term increase in risk at the

[3] The abbreviation used is: BMI, body mass index.

Table 3 Predictors of stromal proportion among all women, controlling for variables relating to parity, and among parous women only

A. All women	Full model[a] $r^2 = 0.23$		Parsimonious model[a] $r^2 = 0.22$	
Predictors	β	P	β	P
Age at biopsy (years)	−0.07	0.74	−0.08	0.68
Menopause (pre/post)	−4.09	0.17	−4.04	0.16
Proliferative without atypia (yes/no)	4.98	0.04	5.11	0.03
Atypical hyperplasia (yes/no)	4.82	0.21	4.71	0.23
Oral contraceptive use (ever/never)	−5.93	0.01	−5.80	0.01
Family history (yes/no)	−1.28	0.67		
Age at menarche (years)	1.41	0.16		
BMI (weight/height^2)	−0.45	0.09	−0.50	0.05
Height in 1976 (inches)	−0.63	0.16	−0.58	0.19
B. Parous women only	**Full model[b] $r^2 = 0.22$**		**Parsimonious model[c]**	
Predictors	β	P	β	P
Time since last birth (years)	−0.39	0.30		
Age at first birth (years)	−0.07	0.83		
Parity (1–9)	−0.30	0.80		
History of breast feeding (yes/no)	1.06	0.70		

[a] Controlling for combined term including parity (0, 1–2, >2), age at first birth (≤24, 25–29, >30 years), and time since last birth in single years.
[b] Controlling for age at biopsy (years), menopausal status, (pre-, post-, missing), interaction between age and menopause, categories of benign breast disease (nonproliferative, proliferative, and atypical hyperplasia), use of oral contraceptives (ever/never), family history (yes/no), age at menarche (years), BMI (weight/height^2), and height (inches).
[c] None of the parity-related variables met the criteria for inclusion in the parsimonious model.

first full-term pregnancy and a decline in the rate of breast tissue aging during the perimenopausal period. Breast cancer arises in ductal epithelial cells (4), and an increase in cellular mass is hypothesized to increase risk of breast cancer (2). We observed a significantly increased proportion of epithelial tissue in parous women for a period ~10 years since last birth, a plausible explanation for the short-term increased risk of breast cancer after childbirth (25–27).

Women with atypical hyperplasia are at higher risk of breast cancer and did indeed have an elevated proportion of epithelial tissue compared with women without proliferative breast disease in our multivariate analysis. However, the association of proliferative disease without atypia with epithelial and stromal proportion was even stronger than that of atypical hyperplasia, although the risk of breast cancer in this group is lower, suggesting that the presence of atypical cells conveys information independent of the number of epithelial cells.

Current use of oral contraceptives is associated with increased breast cancer risk (28). We found no significant effect of oral contraceptives on epithelial proportion but an inverse association of stromal proportion in women who had ever used contraceptives that was strongest among premenopausal women. Additional work is needed to clarify this association, particularly in relation to duration of use. The association of postmenopausal hormone use was in the expected direction, with current and past users having nonsignificantly higher epithelial and stromal proportion than women who had never used postmenopausal hormone therapy, although the number of women for whom we had information on hormone use was small.

Interactions between stroma and epithelium are important for normal development and maturation of the mammary gland (9, 10, 29). Disruption of the reciprocal interaction between epithelium and stroma has been hypothesized to play a role in the carcinogenic process, although it is generally thought that breast cancer arises because of genetic changes in epithelial cells (9). Our data show a significant correlation between the epithelial and stromal tissue in the breast, suggesting that they may influence each other or may be influenced by the same factors; however, it is impossible to distinguish between these possibilities using this study design.

Age, menopausal status, parity, BMI, hormone use, and menstrual cycle phase have been associated with mammographic density (30–37), but there is debate as to whether age or menopausal status is the more important determinant (32). Although mammographic density is related to both stromal and epithelial proliferation (38–40), the far greater proportion of stromal tissue in the breast than epithelial tissue suggests that stromal proliferation is more important than epithelial proliferation (41). We have shown that there are different influences on epithelial and stromal tissue, which may be of relevance because mammography cannot distinguish between these components of breast tissue.

The quantitative measurements of epithelial and stromal tissue developed in this study are highly reproducible and comparable to mammographic studies in terms of inter- and intraobserver reproducibility (13). An important limitation of our study is that the measurement was based on histological slides from a breast biopsy that was directed at the presence of a palpable lump or mammographic lesion, potentially biasing the sampling toward more dense breast tissue. In addition, epithelial and stromal composition has been shown to vary within the quadrants of the breast (20, 21); therefore, a single biopsy may not be representative of the entire breast. Despite these limitations, we were able to detect different influences on epithelial and stromal tissue, and we did control for the presence of histological type of benign breast disease in the multivariate analysis. Russo and Russo (42) observed that lobule distribution in tissue from benign breast biopsies differed from reduction mammoplasty specimens; thus, the generalizability of our results is restricted to the population of women undergoing breast biopsy.

In summary, epithelial and stromal proportion were associated with different factors. Age, menopausal status, type of

benign breast disease, and years since last birth were independently associated with epithelial proportion of benign breast tissue, whereas type of benign breast disease, oral contraceptive use, and BMI were associated with stromal proportion. Increased epithelial proportion after last birth might explain the short-term increase in risk of breast cancer after pregnancy observed in epidemiological studies. The quantitative measurement of epithelial and stromal proportion may be useful for measuring changes in breast composition and may represent intermediate markers of breast cancer risk in studies where breast biopsies are available.

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