Research Article

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Circulating Sex Hormones and Terminal Duct Lobular Unit Involution of the Normal Breast

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Abstract

Background: Terminal duct lobular units (TDLU) are the predominant source of breast cancers. Lesser degrees of age-related TDLU involution have been associated with increased breast cancer risk, but factors that influence involution are largely unknown. We assessed whether circulating hormones, implicated in breast cancer risk, are associated with levels of TDLU involution using data from the Susan G. Komen Tissue Bank (KTB) at the Indiana University Simon Cancer Center (2009–2011).

Methods: We evaluated three highly reproducible measures of TDLU involution, using normal breast tissue samples from the KTB ($n = 390$): TDLU counts, median TDLU span, and median acini counts per TDLU. RRs (for continuous measures), ORs (for categorical measures), 95% confidence intervals (95% CI), and P_{trends} were calculated to assess the association between tertiles of estradiol, testosterone, sex hormone–binding globulin (SHBG), progesterone, and prolactin with TDLU measures. All models were stratified by menopausal status and adjusted for confounders.

Results: Among premenopausal women, higher prolactin levels were associated with higher TDLU counts (RR_{T3vsT1}:1.18; 95% CI: 1.07–1.31; $P_{trend} = 0.0005$), but higher progesterone was associated with lower TDLU counts (RR_{T3vsT1}: 0.80; 95% CI: 0.72–0.89; $P_{trend} < 0.0001$). Among postmenopausal women, higher levels of estradiol (RR_{T3vsT1}:1.61; 95% CI: 1.32–1.97; $P_{trend} < 0.0001$) and testosterone (RR_{T3vsT1}: 1.32; 95% CI: 1.09–1.59; $P_{trend} = 0.0043$) were associated with higher TDLU counts.

Conclusions: These data suggest that select hormones may influence breast cancer risk potentially through delaying TDLU involution.

Impact: Increased understanding of the relationship between circulating markers and TDLU involution may offer new insights into breast carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 23(12); 2765–73. ©2014 AACR.*

Introduction

Terminal duct lobular units (TDLU), the histologic structures of the breast that are responsible for lactation, are the predominant source of breast cancers (1). TDLUs undergo various physiologic changes throughout the life of a woman (e.g., puberty, pregnancy, lactation, and menopause) and involute as women age (2, 3). TDLU involution is a natural physiologic process characterized by reduction in acini counts per TDLU, TDLU span, and a decrease in number of TDLUs (Fig. 1; refs. 4–8). Failure to undergo TDLU involution among women with benign breast disease has been associated with progression to breast cancer, independent of other breast cancer risk factors (9–13). However, the degree of age-related TDLU involution varies widely among women and factors that influence involution are poorly characterized. Identifying biologic factors associated with TDLU involution in the normal breast could help determine the extent to which molecular markers may influence cancer risk through modifying breast histology (14).

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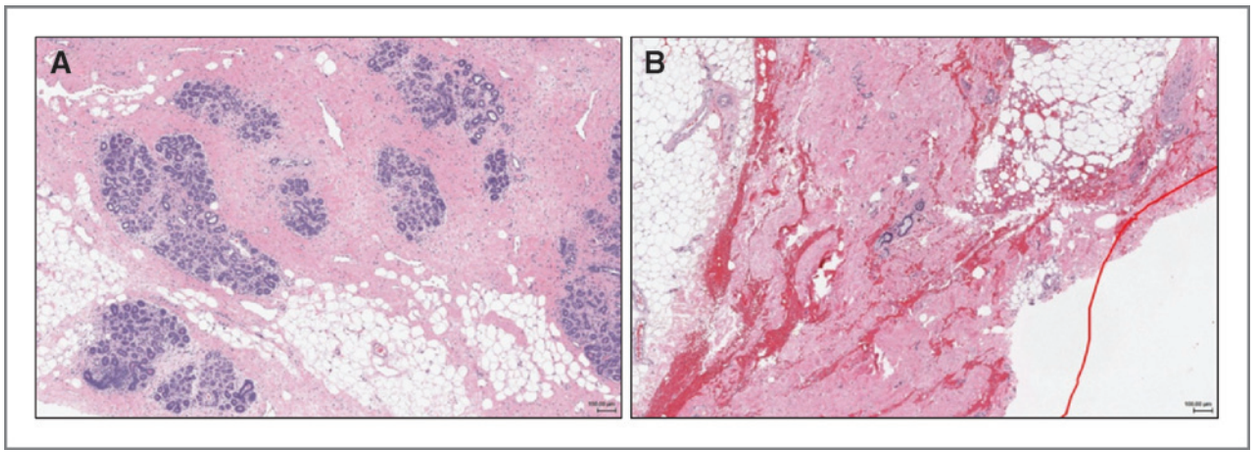

Figure 1. A, a representative H&E image showing a woman with limited TDLU involution, as reflected in the increased number of TDLUs and number of acini within the TDLUs. B, a representative H&E image showing a woman with marked TDLU involution, as reflected by few TDLUs and limited acini content.

We developed three reproducible TDLU involution measures, based on morphometric TDLU assessment (i.e., TDLU counts, span, and acini counts per TDLU), which are inversely associated with TDLU involution (15). Assessment of these measures among women in the Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center (KTB), a unique resource of normal breast tissue samples, blood samples, and clinical data, found older age, parity, and menopausal status among the predominant characteristics associated with TDLU involution.

Our findings from the KTB support a potential hormonal influence in delaying TDLU involution, as several of these factors have been associated with differences in hormone levels (16–19). In addition, increased circulating levels of sex hormones (e.g., estrogens, androgens, and prolactin), and decreased sex-hormone binding globulin (SHBG) levels, have been associated with increased breast cancer risk (20–22). To expand on our understanding of hormonally related breast cancer risk factors, which may influence TDLU involution, we assessed whether circulating levels of hormones are related to TDLU morphometric assessments (i.e., count, span, and acini count per TDLU) among normal breast tissue samples from volunteers in the KTB.

Materials and Methods

Study population

The KTB is a cross-sectional study that recruited 923 volunteer women, without evidence of breast disease, 18 to 84 years of age, from January 10, 2009 through January 22, 2011. Details of this study population and subject ascertainment are described elsewhere (23, 24). All volunteers provided informed consent for the use of their donated specimens and questionnaire data for breast cancer research. Briefly, normal breast tissues and blood samples were collected along with a self-administered questionnaire (including demographic, lifestyle, reproductive, and cancer-related data). All KTB data were collected with the approval of the Indiana University Institutional Review Board and the NIH Office of Human Subjects Research (NIH OHSR #4508).

Of the 923 women, we excluded subjects who were previously diagnosed with cancer ($n = 75$), pregnant ($n = 8$), currently taking menopausal hormones ($n = 36$) or hormonal contraceptives ($n = 209$), and/or missing information on menopausal status ($n = 17$), menstrual cycle phase ($n = 109$), or hormone measurements ($n = 13$). Repeat sample collections from women who were previous tissue donors were excluded ($n = 46$). We also excluded perimenopausal women, defined as women who had not had a period in the last 12 months but had a serum follicle-stimulating hormone (FSH) level of <23 IU/L and estradiol level of >37 pg/ml ($n = 11$) or women who had a period in the last 12 months but had a serum FSH level of >33.4 IU/L ($n = 8$). In addition, one subject with uninterpretable tissue morphology was excluded, leaving 390 subjects in the analytic population. We classified women as postmenopausal if they had an FSH level of ≥23 IU/L and reported a natural menopause (i.e., no menstrual period in the last 12 months that was not due to current oral contraceptive use), a past bilateral oophorectomy before menopause, or a past hysterectomy without bilateral oophorectomy and were older than 55 years of age at the time of interview.

Questionnaire data on various exposures that were evaluated as potential confounders or effect modifiers included age at tissue collection (premenopausal: <55, 55–60, or >60 years; postmenopausal: <30, 30–40, or >40 years), race (White, African American, Hispanic, or other), smoking status (no or yes), body mass index [BMI; underweight/normal: <25, overweight: 25–29.9, or obese: ≥30 weight (kg)/height (m^2)], age at menarche (12, 13, >13 years of age), parity (nulliparous or parous), age at first birth (nulliparous, <25, 25–29, or ≥30 years of age), menstrual cycle phase [follicular: days 1–11; periovulatory: days 12–16; or luteal: days 17–28; calculated from self-reported last menstrual period (LMP) and assuming a

28-day cycle], age at menopause (<45, 45–49, or >49 years), menopause due to bilateral oophorectomy (yes or no), years since menopause (≤5, >5–10, >10–19, or >19), and past menopausal hormone use (never or former).

Morphometric TDLU assessment

Tissue cores were removed from the upper outer quadrant of the breast (left or right) using a standardized technique with a 10-gauge needle. One sample was fixed in 10% buffered formalin, routinely processed to prepare paraffin-embedded blocks, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Digitized images of these sections were used for analysis of percent fat on tissue slide (≤50%, 51–75%, and >75%) and measurements of TDLU involution are described in detail elsewhere (15).

Briefly, we evaluated three TDLU measures, based on morphometric TDLU assessment, and inversely related to TDLU involution: TDLU counts per standardized biopsy section, TDLU span, and categories of acini counts per TDLU (1, <10; 2, 11–20; 3, 21–30; 4, 31–50, and 5, ≥51 acini per TDLU). For women with observable TDLUs, up to 10 TDLUs per section were analyzed for TDLU span and category of acini counts per TDLU. For postmenopausal women, who had much lower acini counts per TDLU (<20 acini per TDLU), we used a semiautomated image analysis tool (25) to obtain an absolute number of acini per TDLU. Median TDLU span, median category of acini counts per TDLU (for premenopausal women), and median acini counts per TDLU (for postmenopausal women) were computed, to provide a single summary measure for each woman.

Hormone measurements

Sex steroid hormones (estradiol, testosterone, and progesterone), SHBG, prolactin, and FSH, were measured in serum. Chemiluminescent immunometric assays and concentrations were calculated by the ADVIA Centaur instrument (Bayer HealthCare) through Quest Diagnostics, Inc. to measure estradiol, prolactin, FSH, and progesterone. SHBG was measured with the Immulite 2000 system (Siemens) and testosterone was measured using liquid chromatography/tandem mass spectrometry (LC/MS-MS). Percent-free estradiol and percent-free testosterone were calculated from measured estradiol, testosterone, and SHBG levels using calculations based on the law of mass action and assuming a constant serum albumin concentration of 4.3 g/dL (26). Blinded quality control samples were placed randomly in each batch and computed coefficients of variation for each hormone were estradiol (10.1%, $n = 18$), testosterone (3.0%, $n = 18$), SHBG (0.9%, $n = 17$), progesterone (6.6%, $n = 12$), prolactin (1.4%, $n = 18$), and FSH (9.1%, $n = 17$). The intraclass correlation coefficient was >99% for all hormone measures. FSH levels were used to define menopausal status and were not an exposure of interest.

All serum samples with hormone levels lower than the limit of detection were recoded as 0.0001. Among premenopausal women, 5 (2.1%) had estradiol levels <7 pg/mL (1 subject missing estradiol levels), 22 (9.4%) had testosterone levels <1 ng/dL (5 subjects missing testosterone levels), and 47 (19.8%) had progesterone levels <0.2 ng/mL. Among postmenopausal women, 37 (24.3%) subjects had estradiol levels <7 pg/mL, 25 (16.7%) had testosterone levels <1 ng/dL (subjects missing testosterone levels), and 121 (79.6%) had progesterone levels <0.2 ng/mL. Approximately 20% of postmenopausal women had detectable levels of progesterone; therefore, progesterone was only assessed dichotomously among postmenopausal women (detectable versus not detectable).

Statistical analysis

All analyses and sensitivity analyses were stratified by menopausal status (premenopausal and postmenopausal), as these populations have differing reference ranges for the measured serum hormone levels (27–29). Frequencies and percentages were used to describe selected characteristics of the study population. Hormone levels were categorized in tertiles, within pre- and postmenopausal populations, for analysis. Covariates that had a significant association with TDLU measures through χ^2 or Kruskal–Wallis tests were included in multivariable models. The final multivariable models only included confounders with a $P < 0.05$ when assessing the relationship between hormones and TDLU measures.

Ordinal logistic regression models analyzing TDLU measures in deciles were used to assess the fit of a Poisson distribution and the Vuong test was used to assess the fit of a zero inflated Poisson (ZIP) distribution (30). To account for the excess zero TDLU counts in the study population, multivariable ZIP regression was used to calculate RRs and 95% confidence intervals (CI) for the relationship between serum hormone levels and TDLU counts. TDLU counts were additionally adjusted for categories of percent fat on tissue slide, as these pathologic measures were significantly associated. Among women with at least one TDLU, multivariable, ordinal logistic regression models were used to calculate ORs and 95% CIs to assess the relationship between hormone levels with median TDLU span and median category of acini counts per TDLU (among premenopausal women), categorized in tertiles to account for non-normal distributions while still having the ability to test for a potential dose–response relationship with hormone levels. As absolute acini counts per TDLU were available for postmenopausal women, multivariable, Poisson regression models were used to calculate RRs and 95% CIs between hormone levels and median acini counts per TDLU. The Wald test was used to assess a linear trend in the relationship between hormone tertile levels and TDLU involution measures. We applied a Bonferroni correction for multiple testing and considered a $P_{trend} < 0.0031$ to be statistically significant (14 tests per outcome), and $0.0031 < P_{trend} \leq 0.05$ as suggestive.

As estradiol, testosterone, and progesterone fluctuate by menstrual cycle phase (27), a sensitivity analysis that stratified models by menstrual cycle phase was

Table 1. Selected characteristics, stratified by menopausal status of women in the KTB

Characteristics	Premenopausal ($N = 238$)	Postmenopausal ($N = 152$)
	n (%)	*n* (%)
Age (mean, SD)	34.8 (9.5)	59.8 (8.7)
Age, y		
<20	14 (5.9)	—
20–29	63 (26.5)	—
30–39	75 (31.5)	2 (1.3)
40–49	78 (32.8)	14 (9.2)
50–59	8 (3.3)	77 (50.7)
≥60	—	59 (38.8)
Race/ethnicity		
White	187 (78.6)	129 (84.9)
African American	8 (3.4)	10 (6.6)
Hispanic	30 (12.6)	5 (3.3)
Other	13 (5.4)	8 (5.2)
Current smoker		
No	213 (90.6)	139 (92.1)
Yes	22 (9.4)	12 (8.0)
BMI (kg/m^2)		
Underweight/ normal (<25)	90 (38.2)	47 (31.3)
Overweight (25–25.9)	73 (30.9)	51 (34.0)
Obese (≥30)	73 (30.9)	52 (34.7)
Age at menarche		
≤12	103 (43.2)	72 (47.7)
13	78 (32.8)	43 (28.5)
>13	57 (24.0)	36 (23.8)
Menstrual cycle phase		
Follicular	108 (45.4)	— (—)
Periovulatory	44 (18.5)	
Luteal	86 (36.1)	
Age at first birth, y		
Nulliparous	112 (47.3)	26 (17.2)
<25	50 (21.1)	51 (33.8)
25–29	42 (17.7)	52 (34.4)
≥30	33 (13.9)	22 (14.6)
Age at menopause, y		
<45	— (—)	23 (15.1)
45–49		41 (27.0)
≥50		57 (37.5)
Unknown		31 (20.4)
Bilateral oophorectomy[a]		
No	— (—)	132 (86.8)
Yes		20 (13.2)
Years since menopause		
<5	— (—)	29 (19.1)
6–8		30 (19.7)
11–20		30 (19.7)
≥20		32 (21.1)
Unknown[b]		31 (20.4)

(Continued in the following column)

Table 1. Selected characteristics, stratified by menopausal status of women in the KTB (Cont'd)

Characteristics	Premenopausal ($N = 238$)	Postmenopausal ($N = 152$)
Past menopausal hormone use		
No	— (—)	76 (50.3)
Yes		75 (49.7)
Family history of breast cancer[c]		
No	191 (80.2)	104 (68.4)
Yes	47 (19.8)	48 (31.6)

NOTE: KTB, Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center.

[a]Menopausal status due to bilateral oophorectomy.

[b]Women with unknown years since menopause either were missing this data ($n = 9$) or were women with hysterectomies prior to menopause and were >55 years of age ($n = 22$).

[c]Women with at least one first-degree relative with a diagnosis of breast cancer.

conducted. Prolactin levels and TDLU measurements were assessed after stratification by parity (nulliparous and parous) as past analyses have shown reductions in prolactin levels associated with parity (31, 32). All analyses were conducted in SAS v9.3 (2011, SAS Institute).

Results

Subject characteristics

The study population was predominantly premenopausal ($n = 238$, 61%) and White ($n = 316$, 81%; Table 1). Among the premenopausal population (mean age 35 years), estimated menstrual cycle phase at sample collection was follicular for 45%, periovulatory for 19%, and luteal for 36% of women. Approximately 47% of the premenopausal women were nulliparous versus 17% in the postmenopausal population. Among the postmenopausal population (mean age 60 years), 47% of women who knew date of menopause were ≥50 years of age at menopause, 13% were menopausal secondary to bilateral oophorectomy, and approximately 50% were former menopausal hormone users.

Median and interquartile range (IQR) of serum hormone levels stratified by menopausal status and menstrual cycle phase are provided in Table 2. Spearman rank correlation coefficients (Supplementary Table S1) and descriptive statistics for TDLU measures by tertiles of hormone levels (Supplementary Table S2) are presented in Supplementary Tables. Multiple associations between serum hormone levels and TDLU counts were found to be suggestive ($0.0031 < P_{trend} \leq 0.05$) and significant ($P_{trend} < 0.0031$), among premenopausal and postmenopausal women (Table 3). Among the subset of women with observable TDLUs (premenopausal:

Table 2. Serum hormone levels by menopausal status among women in the KTB

	Premenopausal												
	Follicular			Periovulatory			Luteal			Postmenopausal			Kruskal–Wallis *P* values
Serum hormone levels	*N*	Median	IQR	*N*	Median	IQR	*N*	Median	IQR	*N*	Median	IQR	Post- vs. premenopausal
Estradiol (pg/mL)	107	58.0	37.0–112.0	44	124.0	54.0–213.5	86	91.0	59.0–141.0	152	15.0	7.0–23.5	<0.0001
Free estradiol (%)	93	2.4	1.9–2.6	38	2.5	2.0–2.8	76	2.2	1.8–2.6	130	2.3	1.9–2.6	0.71
Testosterone (ng/dL)	104	13.0	8.0–22.0	43	19.0	14.0–24.0	86	14.0	7.0–23.0	150	11.0	4.0–16.0	<0.0001
Free testosterone (%)	93	1.4	1.0–1.7	37	1.6	1.0–1.9	76	1.2	0.9–1.6	130	1.3	1.0–1.7	0.76
SHBG (nmol/L)	95	47.0	35.0–80.0	39	41.0	27.0–74.0	77	58.0	38.0–93.0	134	55.0	34.0–81.0	0.97
Progesterone (ng/mL)	108	0.3	<0.2–0.7	44	0.6	0.2–1.6	86	5.7	1.0–8.9	152	—[a]	—	<0.0001
Prolactin (ng/mL)	108	7.6	5.6–10.0	44	8.4	6.0–11.5	86	8.7	6.4–10.7	152	5.2	4.1–7.0	<0.0001

[a]Women with detectable and nondetectable levels of progesterone were compared among postmenopausal women, as 80% did not have detectable levels.

$n = 180$, 76%; postmenopausal: $n = 87$, 57%), no significant associations with median TDLU span or median acini counts per TDLU (categorical and absolute) were observed (Supplementary Tables S3 and S4, respectively).

Premenopausal women

Among premenopausal women, estradiol (RR_{T3vsT1}: 0.88; 95% CI: 0.80–0.97; $P_{trend} = 0.01$) and calculated percent-free estradiol (RR_{T3vsT1}: 0.89; 95% CI: 0.79–1.00; $P_{trend} = 0.05$) levels in the highest tertile, versus lowest,

Table 3. Associations[a] between hormone levels and TDLU counts among women in the KTB

	Premenopausal[b]			Postmenopausal[c]		
Serum hormone levels	*N*	RR (95% CI)	P_{trend}	*N*	RR (95% CI)	P_{trend}
Estradiol	236			152		
T2 vs. T1		0.97 (0.88–1.08)			0.86 (0.69–1.08)	
T3 vs. T1		0.88 (0.80–0.97)	0.01		1.61 (1.32–1.97)	<0.0001
Free estradiol (%)	206			130		
T2 vs. T1		0.87 (0.78–0.98)			1.35 (1.09–1.67)	
T3 vs. T1		0.89 (0.79–1.00)	0.05		0.87 (0.67–1.11)	0.45
Testosterone	232			148		
T2 vs. T1		0.94 (0.85–1.04)			1.18 (0.97–1.44)	
T3 vs. T1		0.97 (0.88–1.08)	0.60		1.32 (1.09–1.59)	0.0043
Free testosterone (%)	205			130		
T2 vs. T1		0.87 (0.78–0.98)			1.35 (1.09–1.67)	
T3 vs. T1		0.89 (0.80–1.00)	0.05		0.86 (0.67–1.11)	0.45
SHBG	210			134		
T2 vs. T1		0.93 (0.84–1.05)			1.54 (1.22–1.93)	
T3 vs. T1		1.04 (0.93–1.16)	0.51		1.14 (0.89–1.45)	0.54
Progesterone	237			148		
T2 vs. T1		0.96 (0.87–1.06)			0.88 (0.71–1.08)	
T3 vs. T1		0.80 (0.72–0.89)	< 0.0001			0.23[d]
Prolactin	237			152		
T2 vs. T1		0.85 (0.76–0.95)			1.28 (1.04–1.58)	
T3 vs. T1		1.18 (1.07–1.31)	0.0005		1.29 (1.04–1.59)	0.02

Abbreviations: T1, tertile one; T2, tertile two; T3, tertile three.

[a]Only results for the Poisson count models from the zero-inflated Poisson models are shown, as no results were significant in the binomial logit models. Only $P_{trend} < 0.0031$ were significant and $0.0031 < P_{trend} \leq 0.05$ were suggestive, after Bonferroni correction.

[b]Adjusted for parity/age at first birth and percent fat on tissue slide.

[c]Adjusted for parity/age at first birth, years since menopause, and percent fat on tissue slide.

[d]RR, 95% CIs, and *P* value for detectable versus not detectable levels of progesterone.

demonstrated suggestive associations with lower TDLU counts (Table 3). A similar association was seen for calculated percent-free testosterone (RR_{T3vsT1}: 0.89; 95% CI: 0.80–1.00; $P_{trend} = 0.05$) but not testosterone (RR_{T3vsT1}: 0.97; 95% CI: 0.88–1.08; $P_{trend} = 0.60$). In analyses stratified by menstrual cycle phase, a suggestive association between follicular estradiol levels and TDLU counts (RR_{T3vsT1}: 1.21; 95% CI: 1.04–1.42; $P_{trend} = 0.03$), but not with periovulatory or luteal estradiol levels, was observed (Supplementary Table S5).

No associations between SHBG levels with TDLU counts were observed (Table 3). Women with progesterone levels in the highest tertile had lower TDLU counts (RR_{T3vsT1}: 0.80; 95% CI: 0.72–0.89; $P_{trend} < 0.0001$), compared with women in the lowest tertile. This association persisted in a sensitivity analysis excluding women >45 years of age, who may have been perimenopausal (data not shown). After stratification by menstrual cycle phase, this association was only observed in the luteal phase (RR_{T3vsT1}: 0.57; 95% CI: 0.13–0.59; $P_{trend} < 0.0001$; Supplementary Table S5). Women with prolactin levels in the highest tertile had higher TDLU counts (RR_{T3vsT1}: 1.18; 95% CI: 1.07–1.31; $P_{trend} = 0.0005$), compared with women in the lowest tertile (Table 3). When stratified by parity, this association was restricted to nulliparous women (RR_{T3vsT1}: 1.64; 95% CI: 1.38–1.94; $P_{trend} < 0.0001$), with no significant association observed among parous women (RR_{T3vsT1}: 0.88; 95% CI: 0.77–1.00; $P_{trend} = 0.03$; Supplementary Table S6).

Postmenopausal women

In the postmenopausal population, women in the highest estradiol tertile were more likely to have higher TDLU counts (RR_{T3vsT1}: 1.61; 95% CI: 1.32–1.97; $P_{trend} < 0.0001$), though associations were not observed with calculated percent-free estradiol levels (Table 3). A suggestive association was observed between testosterone levels and TDLU counts (RR_{T3vsT1}: 1.32; 95% CI: 1.09–1.59; $P_{trend} = 0.0043$), but not with calculated percent-free testosterone. SHBG levels and detectable versus not detectable levels of progesterone were not significantly associated with TDLU counts. Similar to premenopausal women, a suggestive positive association between prolactin and TDLU counts (RR_{T3vsT1}: 1.29; 95% CI: 1.04–1.59; $P_{trend} = 0.02$) was observed. Contrary to premenopausal women, a positive association between prolactin levels and TDLU counts (RR_{T3vsT1}: 1.51; 95% CI: 1.19–1.90; $P_{trend} = 0.0009$) was observed among postmenopausal parous women (Supplementary Table S6). All results were similar after exclusion of former menopausal hormone users (Supplementary Table S7).

Discussion

We observed significant associations between higher TDLU counts, representing less involution, with higher levels of prolactin and lower levels of progesterone, among premenopausal women, and higher levels of estradiol, among postmenopausal women. No significant associations were seen with TDLU span or acini counts per TDLU, which may be due to insufficient power, as these outcomes were only analyzed among women with observable TDLUs (premenopausal: $n = 180$, 76%; postmenopausal: $n = 87$, 57%). Also, these measures may represent earlier stages of involution whereas TDLU counts likely represents final stages of involution (5). Lack of TDLU involution has been associated with increased breast cancer risk (9–11), but relationships between sex hormone levels and TDLU morphometric assessments in the normal breast have not previously been studied. Results of this analysis suggest that hormone levels may act, at least in part, by delaying age appropriate TDLU involution, and thereby leaving a higher quantity of at-risk epithelium.

Our finding that elevated postmenopausal estradiol levels are associated with greater TDLU counts is consistent with the growth promoting role of estrogens in breast development (33) and has been well established as a risk factor for breast cancer among postmenopausal women (18, 34). In the Nurses' Health Study, positive associations between total and percent-free estradiol with breast cancer risk were observed, even when diagnosis was over 15 years after blood collection (35). In addition, estrogen levels have been linked to multiple breast cancer risk factors (e.g., older age, older age at menopause, younger age at menarche, higher BMI, smoking, and alcohol consumption) among postmenopausal women (19). Limited data also suggest positive associations between premenopausal estrogen levels and breast cancer risk (21), but we only observed a suggestive association between higher follicular estradiol levels and elevated TDLU counts.

Higher testosterone levels were suggestively associated with higher TDLU counts among postmenopausal women. Although little is known about the role of androgens in age-related involution, testosterone may influence TDLU counts as a precursor in estrogen production (36, 37). Higher androgen levels have also been associated with breast cancer risk and risk factors (e.g., older age, natural menopause, higher BMI, and smoking) among both postmenopausal (19, 20, 35, 38, 39) and premenopausal women (21, 37, 40, 41). Null associations between SHBG and TDLU counts were consistent with past breast cancer risk studies among premenopausal women (21), but not postmenopausal women where inverse associations have been observed (20).

Progesterone is related to proliferation of acini and stromal elements, particularly in relationship to development and pregnancy, with epithelial cell proliferation at its highest during the luteal phase of the menstrual cycle (27). We found that higher progesterone concentrations during the luteal phase were inversely related to TDLU counts, though we did not have data on menstrual cycle length. This relationship may be skewed by women who are potentially perimenopausal and/or anovulatory, characterized by luteal insufficiency (42); however, this inverse association persisted in analysis restricted to women <45 years of age. Our findings are not consistent

with previous studies of progesterone levels and breast cancer risk that have mostly found null associations (21, 37). A positive association between progesterone and TDLU counts might be hypothesized, as progesterone stimulates lobular cell proliferation and would be consistent with an increased breast cancer risk observed among postmenopausal estrogen plus progestin menopausal hormone users versus estrogen only menopausal hormone users in past analyses (43). However, more recent studies have not found an increased risk for women using progestins that are structurally identical to natural progesterone (i.e., micronized progesterone; refs. 44–46). In addition, emerging evidence suggests progesterone may inhibit proliferation in normal breast tissue and stimulate proliferation in tumorous breast tissue, due to possible differences in progesterone metabolism (47).

Prolactin has been shown to be involved in the proliferation and differentiation of lobular acini tissue during pregnancy and lactogenesis, but is also present throughout normal breast development (7, 48). Higher prolactin levels have previously been associated with breast cancer risk and risk factors such as nulliparity and higher BMI among postmenopausal women (17, 32, 49–52); and nulliparity, later age at first birth, and family history of breast cancer among premenopausal women (17, 31, 32, 51, 53). However, the association between prolactin and breast cancer risk was not significant among premenopausal women in extended follow-up analyses (22, 52). Our analysis found strong associations between higher prolactin levels and higher TDLU counts among nulliparous premenopausal women and parous postmenopausal women. We were not able to analyze this association among postmenopausal nulliparous women due to a small sample size ($n = 26$). The inability to identify an association among parous premenopausal women is likely due to our limited data on reproductive history (i.e., data for all past pregnancies, time since last pregnancy, and date of birth for each live pregnancy were not collected) and are needed to further understand the relationship between prolactin and TDLU involution.

Strengths of this study include the use of reproducible morphometric assessments of TDLU involution and access to a large sample of normal breast tissues from the KTB, representative of women in all stages of involution (18–84 years of age). Additional strengths are the standardized collection, processing, and storage of tissue and blood samples, which permitted us to explore, for the first time, the relationship between measures of TDLU involution and endogenous hormones. Our study is also representative of a healthy population as it utilized normal breast tissue samples, unlike past studies of TDLU involution that utilized collections of breast tissue from biopsies and mammoplasties from women in a clinical setting with abnormal or high risk screening results (e.g., mammogram, ultrasound, or breast exam; ref. 54).

The cross-sectional study design limits our ability to determine temporality of hormone associations with TDLU involution; hence, future analyses should replicate these finding in longitudinal samples. Time of blood draw varied and limited the ability to adjust for diurnal variations in hormone levels, but these issues would likely bias estimates towards the null and underestimate the association between hormonal levels and TDLU involution. Furthermore, blood draws were not coordinated with menstrual cycle phase, and details of cycle length and regularity were unknown; therefore, phase was calculated from self-reported LMP and based on the assumption that all women followed a 28-day cycle. In addition, utilizing more sensitive assays (e.g., LC/MS-MS) in future assessment of estradiol and progesterone levels, in postmenopausal women, could provide further insights into hormonal determinants that may influence TDLU involution levels.

As circulating hormone levels and TDLU involution are dynamic biomarkers, particularly among premenopausal women, future research based on repeat measures may clarify relationships. Furthermore, analysis of biologically active metabolites of sex steroid hormones have substantial differences in activity of cell proliferation, and the effect of this variation might provide further insight into the hormonal pathways involved in TDLU involution. Overall, these data suggest that select hormones are associated with TDLU counts and may influence breast cancer risk by delaying TDLU involution. Therefore, developing an increased understanding of the relationship between hormone levels and TDLU involution may offer new insights into breast carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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