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First pregnancy characteristics, postmenopausal breast density, and salivary sex hormone levels in a population at high risk for breast cancer

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ABSTRACT

Background: It remains unknown if later life breast cancer risk as determined by reproductive history is mediated by postmenopausal breast density and/or sex steroid levels.
Methods: Increased breast density is a strong surrogate for future breast cancer risk. A cross-sectional study with a longitudinal follow-up for breast health outcomes evaluated women without breast cancer ($n = 1023$; 682 = parous), drawn from a high risk postmenopausal population, with questionnaire- reported reproductive histories. The questionnaire was linked to prospective screening mammogram breast density measurements, and saliva biospecimens that were used to assess sex steroid hormone levels.
Results: Expected age- and postmenopause- related declines in salivary estradiol (E), progesterone (P), dehydroepiandrosterone (DHEA) and testosterone (T) levels were observed. This was most pronounced for DHEA and T, which were also the only postmenopausal hormone levels significantly associated with any reproductive characteristics: parity and breast feeding for DHEA, and age-at-first birth for T. Postmenopausal breast density was borderline significantly lower with parity and higher body mass index (BMI). After multivariate analysis, T was the only hormone level to retain any association (negative, $p < 0.05$) with breast density.
Conclusions and general significance: While reproductive characteristics, in particular parity, generally demonstrated independent associations with postmenopausal breast density and E, P and DHEA levels, T levels showed concordant inverse associations with age-at-first birth and breast density. These findings suggest that reproductive effects and later life salivary sex steroid hormone levels may have independent effects on later life breast density and cancer risk.

1. Introduction

Reproductive history, and in particular age at first birth, has been repeatedly demonstrated to be associated with breast cancer risk [1]. However, there is a lack of understanding of the mechanisms by which pregnancy confers changes in breast cancer risk, thus limiting the extent to which these findings can be translated into interventions for prevention. Two mechanistic hypotheses include: i. Persistent changes in the hormonal milieu, and ii. Permanent morphological and gene expression changes imprinted by pregnancy-induced mammary gland differentiation. There is support for each of these hypotheses from epidemiological [1,2], observational and intervention research [3], and animal studies [4–6]. Either of these hypotheses may be manifested by changes in later life breast density.In addition to age-at-first full-term birth, epidemiological evidence supports an association between breast cancer risk and prior pregnancy characteristics including pre-term birth, preeclampsia, multi-fetal gestation and small placental weight [1,7,8]. Mammographic density has a strong relationship to breast cancer risk [9,10]. Reproductive history has also been reported to be related to breast density, with increased density associated with pre-term birth, nulliparity, and late age-at-first birth [8,11–13]. These findings appear strongest for first pregnancies, although many remain controversial [10]. Confirmation of these findings could provide evidence that pregnancy characteristics influence breast cancer risk through hormonally mediated changes in the structure of the breast. There is however, conflicting evidence for a link between pregnancy, hormone levels and breast cancer. A secondary analysis of the Nurses Health Study showed a relationship between postmenopausal serum

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hormone levels and parity, as well as age-at-first birth [14]; but other studies have shown no association between circulating sex hormones and parity [15–17]. In postmenopausal women, circulating sex steroid hormone levels are strongly associated with breast cancer risk [16,17], but any association of these hormone levels with breast density remains uncertain.

To address the question of whether early life reproductive factors, known to be associated with later life breast cancer risk, either mediate or moderate postmenopausal breast density and/or salivary sex steroid levels, we performed a cross-sectional analysis drawn from the larger Marin Women's Study (MWS). Postmenopausal women without breast cancer ($n = 1{,}023$; 682 = parous), and their self reported lifestyle and reproductive characteristics (including first pregnancy events), were examined along with screening mammogram breast density measurements. Self obtained saliva biospecimens were used to assess sex steroid hormone levels (estradiol, progesterone, dehydroepiandrosterone, testosterone). Saliva measurements reflect (but are not necessarily equal to) free plasma or serum concentrations of various steroidal hormones, certain growth factors, and many drugs if they are capable of being transferred by either intracellular (e.g. diffusion) or extracellular (e.g. ultrafiltration) mechanisms. Thus, numerous studies of saliva-based diagnostics have established that clinically relevant analyte concentrations in saliva correlated with tissue fluid levels and can be used either for drug monitoring or to evaluate endocrine function, in particular circulating (unbound) levels of lipid-soluble steroids like cortisol, aldosterone, dehydroepiandrosterone, testosterone, progesterone, and estradiol [18].

2. Materials and methods

2.1. Marin Women's Study (MWS) population and measurements

This study was conducted within the context of the MWS. Marin residents were recruited through mammography facilities in Marin County and San Francisco which are included in the San Francisco Mammography Registry (SFMR), one of the seven registries comprising the National Cancer Institute Breast Cancer Surveillance Consortium. This study was approved by the Marin General Hospital and Kaiser Permanente Northern California Institutional Review Boards, and all participants provided informed consent to fully participate in the study. Primary data collection in the MWS included self-reported information via a detailed questionnaire and saliva samples collected from consenting women. Secondary data were obtained by linkage with the SFMR on volumetric compositional breast density and breast cancer case status, as well as family history, weight and height. The MWS has been previously described and characterized [12]. To date, 13,365 women have been enrolled in the MWS and completed the questionnaire. Of these, about 85% also consented to saliva donation, and 70% completed the process of donation as instructed and produced biobanked specimens.

2.2. Questionnaire components

The questionnaire was filled out by all consenting women as their entry point into the MWS. It included detailed questions about reproductive history, life course socioeconomic data, alcohol use, and medication use, including NSAIDs, which can affect endogenous levels of steroids like DHEA [19]. Additional questions about well-established risk factors included exogenous hormone exposures, and history of previous breast procedures. Reproductive factors included age at menarche and menopause, and specific pregnancy-related questions included parity, age-at-first birth, infertility and treatment for infertility, duration of breast feeding, birth weight of children, preterm birth, pregnancy weight gain, and pregnancy related hypertension.

2.3. Calibrated mammographic density

One of the novel features of this study is the measure of breast density as % fibroglandular volume (%FGV), by the method of single-energy X-ray absorptiometry (SXA). This method uses a calibration phantom of the same thickness as the compressed breast, circumventing some of the problems associated with other breast density measures, such as subjectivity and a lack of absolute reference standards [20]. This study used the first generation calibration phantom (Gamma). Initial results on over 8600 women showed that SXA is precise and accurate when using reference phantoms, and inversely correlated with age, BMI and menopausal status; it is also positively associated with breast cancer risk [21,22]. %FGV data were obtained from the SFMR through a cooperative agreement upon linkage to MWS data for all consenting women.

2.4. Saliva collection and steroid hormone assay

Saliva samples were collected to assess steroid hormone levels, as saliva testing represents a cost effective approach to screening large populations [18]. Sex steroids were measured from cryobanked saliva after precipitating out all cell and particulate components. At the time of entry into the MWS, women were asked on the questionnaire if they were willing to donate a saliva specimen. Those who consented were sent a kit in the mail. Returned specimens were bar coded, logged and cryobanked. In total, 8598 saliva samples have been processed. Processed supernatants were sent to Aeron Biotechnology, Inc. (San Leandro, CA) for radioimmunoassay (RIA) of dehydroepiandrosterone (DHEA), estradiol (E), progesterone (P) and testosterone (T). The entire MWS sample set submitted for analysis of these four steroid hormones ($n = 1784$) were compared to results from an independent contemporary cohort of female samples randomly submitted for commercial analysis, to confirm expected age-specific changes in the hormone levels (Fig. 1). Criteria for inclusion in the MWS saliva analysis required submission of a non-bloody early morning saliva sample of >3 ml volume, following at least 8 h of fasting. Samples from post-menopausal women required attesting to an absence of menses for at least one year.

2.5. Statistical analyses

The analytic sample comprised 1023 postmenopausal women not taking exogenous hormones who had an analyzable saliva sample for hormone levels and questionnaire data on the variables included in the model. Distributions of the salivary hormone levels were examined against a reference range of postmenopausal women to verify that levels were consistent with the known ranges for this population. Multivariable linear regression analyses were constructed using Stata 11.2 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP) to examine the associations between salivary hormone levels, %FGV and reproductive factors, controlling for relevant confounders. The models employed robust regression using iteratively reweighted least squares to minimize the effects of outliers. To examine the association between salivary hormone levels and known or suspected confounders, a separate model was constructed for each hormone and included a base set of confounding variables. The base set of variables included continuous current age, BMI, hours of weekly moderate or vigorous exercise, number of alcoholic drinks consumed per day, parity, and age at menopause, as well as race (Black, White, Asian, Hispanic, Other), education (high school or less, some college, college degree or higher), use of complementary and alternative medicines (CAMS—or natural nonprescription hormone medications) (yes/no), and age at menarche (10 or younger, 11–14, 15+). One model was constructed for the entire population examining the reproductive factors of parity, age at menarche, and age at menopause. Another model restricted to parous women was examined using a wider range of reproductive factors related to the first pregnancy including weeks gestation, high

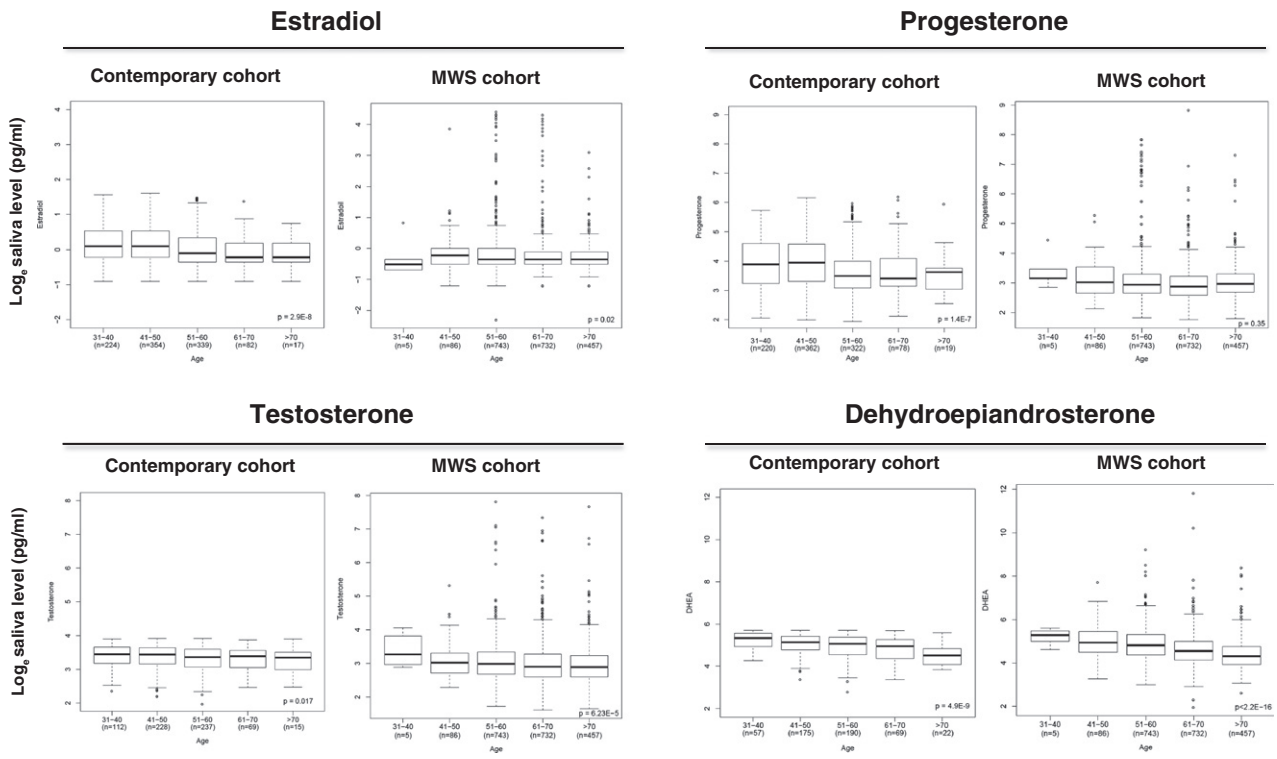

Fig. 1. Age-specific salivary sex steroid hormone levels in the Marin Women's Study (MWS) cohort. Early morning saliva samples ($n = 1784$) collected, cryobanked and processed in compliance with the MWS protocol, as described in Methods, were analyzed by RIA for levels of estradiol (E), progesterone (P), testosterone (T) and dehydroepiandrosterone (DHEA). The age (decade) distribution of log-transformed hormone values is box plotted as shown; and the age-specific sex steroid hormone levels from the MWS samples are shown in relation to a geographically independent, contemporary cohort of US females (not part of the MWS) who provided saliva samples by the same collection protocol, identically processed and analyzed (Aeron Biotechnology). Age-specific changes in hormone levels were tested for significance (p-values) by analysis of trends. A subset of these MWS saliva samples collected from postmenopausal women ($n = 1023$) were used for the study comparison with reproductive characteristics and postmenopausal breast density.

blood pressure, gestational weight gain, age-at-first full term birth, birth weight, and duration of breast feeding. Prior to regression, hormone levels were log transformed and % FGV was square root transformed to normalize the distribution. Women were excluded from all analyses if they had a history of breast cancer, if their first birth was multiple gestation, if they had used antiestrogens in the last five years, if they had a history of ovariectomy, or if any of the variable data was missing from the questionnaire. In the model where %FGV was the dependent variable, we also controlled for family history of breast cancer (first degree relative), hormone use near the time of the mammogram, and a measure of the number of days between saliva donation and %FGV measurement. Models in which one of the four assayed hormone levels was the dependent variable included the base set of confounding variables plus batch, number of hours fasting, and time of saliva donation. Given the numerous comparisons being made, borderline significant findings are not highlighted in the results section.

3. Results

3.1. The Marin Women's Study (MWS) biospecimens and saliva steroid levels

The results of the 1784 saliva supernatants submitted for commercial analysis of steroid hormone levels are presented in Fig. 1. The age specific hormone levels of the MWS cohort appear generally concordant with those of an independent and contemporary cohort of women, in which expected age and postmenopausal hormonal declines are apparent. In both these female cohorts, the most significant age-related hormonal decreases were noted in DHEA and testosterone levels (Fig. 1). Many of the elevated estradiol and progesterone levels in MWS study subjects over age 50 illustrated in this figure reflected their reported use of HRT; these study samples were excluded from the subsequent analysis of postmenopausal subjects, resulting in a final postmenopausal set of 1023 saliva samples for hormone analysis.

Table 1
Covariate distribution ($n = 1023$).

Characteristic	Mean (SD)
%FGV	28.49 (16.59)
Parity	1.63 (1.29)
Number of alcoholic drinks per day	0.91 (.99)
BMI	24.76 (4.69)
Hours of strenuous and moderate exercise per week	8.99 (3.94)
Age of menopause (among menopausal women)	50.81 (5.74)
Current age (years)	62.80 (8.02)
Characteristic	Percent
Taking CAMS	4.50%
Smoking	
Never	46.92%
Current	3.03%
Former	50.05%
Education	
HS or less	4.69%
Some college	26.20%
College or more	69.11%
Race	
White	93.16%
Black	0.39%
Asian	2.74%
Other	1.76%
Hispanic	1.96%

3.2. Postmenopausal study sample summary characteristics (breast density, reproductive parameters, hormone levels)

The study sample ($n = 1023$) represents the subset of postmenopausal study subjects submitting saliva samples eligible for hormone analysis (Table 1). The mean %FGV in the analysis population was 28.49. The majority of the population is white, has a college degree, a normal BMI, and is on average 11.8 years postmenopausal.

Table 2 presents the distribution of reproductive characteristics for this postmenopausal MWS cohort. Nearly one quarter (24.8%) of women had a first birth at age 30 years or older. Pregnancy induced hypertension was reported by 4.47% of the population, and high and low birth weight were reported by 9.14% and 5.49% of the cohort, respectively. Ninety-two percent (92%) of the respondents reported that their first birth was full term, with a mean 29.65 pound weight gain. On average, respondents reported 6.34 months of breastfeeding after this first birth.

The distribution of hormone levels (geometric means) is presented in Table 3. Mean levels for the analysis populations were within the reference range for postmenopausal women for DHEA, estradiol, progesterone and testosterone (109.56, 0.77, 20.32, and 19.87, respectively).

3.3. Multivariate analyses

A multivariate model examining the reproductive factors of parity, age at menarche and age at menopause is presented in Table 4. In this set of models, the only association between one of the hormone levels and parity was the positive association with DHEA ($p = 0.01$). Age was significantly negatively associated with levels of DHEA and testosterone ($p < 0.001$). BMI was significantly associated with DHEA ($p = 0.002$), as was weekly exercise ($p = 0.04$) and current smoking ($p = 0.01$). BMI was also significantly associated with estradiol ($p = 0.001$). Use of complementary and alternative medicine (CAMS) was significantly associated with progesterone levels ($p = 0.04$).

Table 5 shows associations between hormone levels and characteristics of first birth in the postmenopausal subset of parous women ($n = 682$). When controlling for all other factors in the model, including levels of the three other study hormones, breast feeding was significantly positively associated with DHEA levels ($p = 0.04$). No other reproductive factors were significantly related to DHEA. The proportion of the variability in DHEA explained by this model was 38% due in large part to the inclusion of estradiol, progesterone and testosterone as variables; an analogous model that did not include simultaneous control for the other three hormones had an R squared value of 10% (data not shown). There were no significant associations between reproductive factors and estradiol or progesterone. The model for estradiol explained 19% of the variability in estradiol, while the model for progesterone explained 20% of the variability in progesterone. Thirty nine percent (39%) of the variability in testosterone was explained by this model.

The results of the multivariate regression model of hormone levels on %FGV are shown in Table 6. The only hormone significantly associated with %FGV was testosterone, which showed a negative association with %FGV ($p = 0.04$). BMI was also significantly, negatively associated with %FGV ($p < 0.001$). Asian women had significantly higher %FGV than white women ($p = 0.02$) even after control for the other model variables.

Table 2
Distribution of reproductive characteristics ($n = 1023$).

Characteristic	Percent
Age at first birth	
Nulligravid	16.42%
Nulliparous	10.75%
<20	2.93%
20–29	45.06%
30–34	15.25%
35+	9.58%
Menarche	
10 or younger	4.99%
11–14	86.61%
15+	8.41%
First pregnancy characteristic in parous women	Percent
Birthweight	
Low	5.49%
High	9.14%
Normal	85.37%
Pregnancy high blood pressure (%)	4.47%
Weeks gestation	
38+	92.27%
36–37	6.51%
≤35	1.22%
First pregnancy characteristic in parous women	Mean (SD)
Pregnancy weight gain (pounds)	29.65 (11.70)
Months of breastfeeding (first child)	6.34 (7.03)

Table 3
Mean levels of salivary hormones (postmenopausal women, $n = 1023$).

	Geometric means (pg/ml)	Reference range (postmenopausal women)
DHEA	109.56	33–200 (age-specific)
Estradiol	0.77	<1.5
Progesterone	20.32	<50
Testosterone	19.87	11–35 (age-specific)

4. Discussion

4.1. Parity, postmenopausal steroid hormone associations, and breast cancer risk

In this study population of 1023 postmenopausal women in the MWS, parity was positively associated with only DHEA. No other salivary hormones were significantly related to parity in this group. When these associations are taken without adjusting for other hormones, it is hard to determine the extent to which postmenopausal DHEA influences breast cancer risk in this population. Postmenopausal testosterone levels have clearly been linked to an increased risk of developing hormone receptor positive breast cancer [23], but such evidence relating to DHEA has not been as convincing. Earlier prospective case control studies have shown that postmenopausal DHEA levels correlate positively with breast cancer risk [24]. However, in a more recent analysis from the Nurses' Health Study of endogenous hormone levels and postmenopausal breast cancer risk wherein significantly positive associations with risk were shown for estradiol and testosterone levels (RR = 1.3 and 1.29, respectively), the weaker increase in breast cancer risk seen with sulfated DHEA (RR = 1.15) became non-significant upon stepwise regression analysis [23]. As a metabolic precursor to both androgens and estrogens, DHEA is produced in the adrenals, gonads and brain; and even much later in life DHEA remains the most abundant of all circulating sex steroids, as shown in the current study (Table 3). Although a weak partial agonist of the androgen receptor (AR) and both forms of estrogen receptor (ERalpha and ERbeta), the higher circulating levels of DHEA over E2 and T do not come close to compensating for its much weaker AR and ER binding affinities. Therefore, consistent with the current controversy over whether DHEA enhances or reduces the risk of breast (or prostate) cancer, not to mention the fact that sex steroid receptor-independent effects of DHEA have also been reported that could alter mammary gland susceptibility to tumorigenesis [25], it is not possible to conclude that our observed association between parity and DHEA is at all linked to postmenopausal breast cancer risk. Of note, DHEA levels can also be induced by vigorous exercise and caloric

Table 4
Linear regression model: salivary hormone levels in postmenopausal MWS women with hormone measurement and model variables ($n = 1023$).

	Log DHEA	Log estradiol	Log progesterone	Log testosterone
	Beta coefficient (95% CI)	Beta coefficient (95% CI)	Beta coefficient (95% CI)	Beta coefficient (95% CI)
Reproductive factors				
Parity (0–5)	.04 (.01, .07)*	.01 (−.01, .02)	−.001 (−.02, .02)	.01 (−.02, .03)
Menarche (vs. ≤10)				
11–14	.07 (−.11, .26)	−.0002 (−.10, .10)	.07 (−.06, .20)	.10 (−.04, .24)
15+	.02 (−.06, .10)	−.04 (−.17, .08)	.11 (−.05, .27)	.05 (−.12, .23)
Age of menopause	.0004 (−.01, .01)	−.001 (−.004, .003)	−.002 (−.01, .003)	.004 (−.002, .01)
Other factors				
Age (years)	−.02 (−.03, −.02)*	−.001 (−.004, .002)	.001 (−.003, .004)	−.01 (−.01, −.01)*
CAMS use	.04 (−.16, .23)	.04 (−.07, .14)	.14 (.003, .28)*	.05 (−.10, .20)
BMI	.01 (.005, .02)*	.01 (−.003, .01)*	−.01 (−.01, .001)	.02 (.01, .02)
Alcohol consumption	.02 (−.02, .06)	.01 (−.02, .03)	−.03 (−.06, .002)**	.03 (−.002, .06)**
Weekly exercise	.01 (.001, .02)*	.001 (−.004, .01)	−.001 (−.01, .01)	.003 (−.005, .01)
Smoking (versus never)				
Current	.31 (.07, .55)*	−.10 (−.23, .03)	.05 (−.12, .21)	.11 (−.08, .29)
Former	.02 (−.06, .10)	.01 (−.03, .06)	.02 (−.04, .08)	.04 (−.02, .11)
Race (vs white)				
Black	.09 (−.55, .73)	−.22 (−.57, .12)	.20 (−.25, .65)	−.18 (−.67, .31)
Asian	−.10 (−.34, .15)	.02 (−.12, .15)	−.04 (−.22, .13)	.01 (−.18, .20)
Other	−.16 (−.46, .14)	−.02 (−.18, .15)	.01 (−.21, .22)	−.20 (−.43, .03)**
Hispanic	−.22 (−.51, .07)	.01 (−.15, .16)	.10 (−.11, .31)	−.16 (−.38, .07)
Education (versus HS or less)				
Some college	.05 (−.15, .25)	.02 (−.09, .13)	−.12 (−.26, .03)	−.05 (−.21, .10)
College graduate	.02 (−.17, .21)	.01 (−.09, .12)	−.11 (−.24, .03)	−.05 (−.20, .10)
R^2	.09	.04	.02	.04

Controlled for batch, hours fasting, and time donated. Models do not include simultaneous control for other three hormones.
** Denotes borderline significance ($p < 0.1$).
* Denotes statistical significance ($p < 0.05$).

Table 5
Linear regression model: salivary hormone levels in parous postmenopausal MWS women with hormone measurement and model variables ($n = 682$).

	Log DHEA	Log estradiol	Log progesterone	Log testosterone
	Beta coefficient (95% CI)	Beta coefficient (95% CI)	Beta coefficient (95% CI)	Beta coefficient (95% CI)
Reproductive factors				
Parity (number: 1–5)	.04 (−.01, .08)	.01 (−.01, .04)	.00004 (−.04, .04)	−.003 (−.04, .03)
Weeks gestation (versus 38+ weeks)				
36–37 weeks	.09 (−.08, .26)	−.03 (−.12, .07)	−.02 (−.15, .11)	−.04 (−.16, .08)
<35 weeks	.04 (−.31, .38)	.04 (−.16, .24)	.001 (−.27, .27)	.10 (−.15, .34)
High blood pressure (vs. no)	−.12 (−.32, .08)	−.03 (−.14, .09)	.10 (−.05, .26)	.01 (−.13, .16)
Gestational weight gain (lbs)	.0005 (−.003, .004)	−.002 (−.004, .001)	.001 (−.002, .004)	−.0001 (−.003, .003)
Age at first birth (vs. <20)				
20–29	.14 (−.07, .36)	.02 (−.11, .14)	−.07 (−.23, .10)	−.14 (−.29, .02)**
30–34	.12 (−.11, .35)	.02 (−.11, .16)	−.07 (−.25, .11)	−.09 (−.25, .07)
35+	.12 (−.12, .37)	−.01 (−.15, .13)	−.05 (−.24, .14)	−.08 (−.25, .10)
Birthweight (vs. normal)				
Low	.09 (−.09, .28)	−.03 (−.13, .08)	.02 (−.13, .16)	−.09 (−.23, .04)
High	.02 (−.12, .16)	.02 (−.06, .11)	.08 (−.03, .19)	−.02 (−.12, .08)
Breastfeeding (months)	.01 (.0003, .01)*	.0004 (−.003, .004)	−.003 (−.01, .002)	−.0004 (−.005, .004)
Menarche (vs. ≤10)				
11–14	.01 (−.18, .20)	.02 (−.09, .13)	−.02 (−.17, .13)	.09 (−.04, .23)
15+	−.03 (−.27, .20)	−.03 (−.17, .10)	.06 (−.12, .25)	.07 (−.09, .24)
Other factors				
Age (years)	−.02 (−.03, −.01)*	−.0004 (−.004, .003)	.01 (.004, .01)*	.004 (−.0005, .01)**
CAMS use	−.11 (−.32, .10)	−.02 (−.14, .11)	.20 (.03, .36)*	−.18 (−.33, −.03)*
BMI	.005 (−.01, .01)	.004 (−.002, .01)	−.01 (−.02, −.01)*	.01 (.002, .02)*
Alcohol consumption	.03 (−.01, .07)	−.01 (−.04, .02)	−.01 (−.04, .03)	.02 (−.01, .06)
Weekly exercise	.01 (−.003, .02)	−.002 (−.01, .004)	.00003 (−.01, .01)	.004 (−.004, .01)
Smoking (versus never)				
Current	.23 (−.01, .47)**	.04 (−.10, .18)	−.21 (−.40, −.02)*	−.001 (−.17, .17)
Former	−.05 (−.13, .04)	.03 (−.02, .08)	.01 (−.06, .08)	.002 (−.06, .06)
Race (vs white)				
Black	.86 (−.17, 1.88)	−.01 (−.61, .60)	−.28 (−1.09, .53)	−.41 (−1.14, .33)
Asian	−.09 (−.32, .15)	−.001 (−.14, .14)	.02 (−.16, .21)	.0002 (−.17, .17)
Other	−.08 (−.40, .24)	.04 (−.14, .23)	.14 (−.11, .39)	−.12 (−.34, .11)
Hispanic	−.13 (−.40, .15)	.08 (−.08, .25)	.21 (−.01, .42)**	−.08 (−.28, .11)
Education (versus HS or less)				
Some college	.05 (−.15, .24)	.01 (−.10, .13)	−.14 (−.29, .01)**	−.03 (−.16, .11)
College graduate	.01 (−.18, .20)	−.01 (−.12, .10)	−.12 (−.27, .03)	−.02 (−.15, .12)
R^2	.38	.19	.20	.39

Controlled for batch, hours fasting, and time donated. Includes simultaneous control for other hormones.
** Denotes borderline significance ($p < 0.1$).
* Denotes statistical significance ($p < 0.05$).

restriction sufficient to achieve a lean body mass. Since vigorous exercise and low BMI are clearly associated with decreased postmenopausal breast cancer risk, higher DHEA levels in the MWS population could actually correlate with lower breast cancer risk by acting as a surrogate biomarker for risk-reducing exercise and lower BMI in this population.

4.2. First pregnancy association with postmenopausal hormone levels and mammographic density

DHEA was significantly associated with breastfeeding among parous women, but among this group of parous women, the number of births (parity) was not significantly associated with DHEA. The only other association between a reproductive factor and hormone level among parous women was the borderline significant negative association between testosterone and age-at-first birth between ages 20 and 29 (compared to birth before age 20). The postmenopausal hormone levels best associated with pregnancy characteristics (DHEA, testosterone) did not show comparable associations with postmenopausal mammographic density. This observation may mean that pregnancy itself induces early, persistent, and protective morphologic changes in the breast reflected in postmenopausal breast density, but by mechanisms other than long lived hormonal changes. In this fashion, first pregnancy characteristics and postmenopausal hormone levels would be expected to influence breast cancer risk independently.

4.3. Reproductive characteristics and postmenopausal mammographic density

Given the strong association between breast cancer risk and mammographic density, whether measured by conventional BIRADS (Breast Imaging Reporting and Data system) classification or more modern SXA quantitation of %FGV (as reported here), there has been continuing interest in determining either correlative or causative links between mammographic density and breast cancer risk although, to date, such biological and genetic links remain largely unresolved [10]. Clear associations between changes in breast density and increasing age, higher BMI, and exogenous hormone (e.g. combined E + P replacement therapy) or anti-hormone (e.g. antiestrogen) use have spurred epidemiologic studies seeking other associations consistent with long term breast hormonal exposure. So far, meta analyses of these studies indicate no consistent or significant associations between postmenopausal breast density and age-at-first birth, breast feeding, or other reproductive characteristics (after adjustment for age and BMI) other than parity [10]. We observed that postmenopausal breast density was borderline significantly lower with parity (as well as with higher BMI), but not with age at menarche, first birth, or menopause. Hence, while our findings appear to be in complete agreement with many other epidemiologic studies, they do not implicate long term hormonal exposure and do not shed any additional light on the partially protective effect of parity on postmenopausal breast density.

Table 6
Linear regression model: %FGV in parous postmenopausal women ($n = 636$).

	%FGV	
	Beta coefficient	(95% CI)
Reproductive factors		
Parity (number: 0–5)	−.07	(−.15,.01)**
Menarche (vs. ≤10)		
11–14	−.28	(−.73,.17)
15+	−.18	(−.72,.36)
Age of menopause	.01	(−.01,.02)
Salivary hormone levels		
DHEA	.08	(−.08,.24)
Estradiol	.09	(−.11,.29)
Progesterone	.05	(−.10,.21)
Testosterone	−.24	(−.45, −.03)*
Other factors		
Age (years)	−.01	(−.02,.01)
Hormone use at mammogram	−.44	(−.95,.08)**
BMI	−.20	(−.22, −.18)*
Race (vs white)		
Black	.82	(−.52, 2.16)
Asian	.62	(.10, 1.15)*
Other	.27	(−.50, 1.05)
Hispanic	.34	(−.28,.95)
Education (versus HS or less)		
Some college	−.09	(−.51,.34)
College graduate	.01	(−.39, 41)
Weekly exercise	−.003	(−.03,.02)
Alcohol consumption	−.01	(−.10,.09)
Smoking (versus never)		
Current	−.06	(−.62,.49)
Former	.03	(−.15,.22)
First degree relative with breast cancer	.09	(−.13,.30)
R^2	.33	

** Denotes borderline significance ($p < 0.1$).
* Denotes statistical significance ($p < 0.05$).

4.4. Study strengths and weaknesses

This study has a number of important strengths, including a relatively large sample size, a novel measure of breast density, and the availability of information on a wide variety of reproductive characteristics and other breast cancer risk factors.

The primary limitation in this study is the use of self-reported data for reproductive history and early life risk factors such as age at menarche. Though it is possible that women may not accurately recall information about their first pregnancy, particularly if it occurred in the distant past, we would expect that they would accurately recall the major events including their age when they gave birth, and whether they breast-fed. To the extent that misclassification of exposures is present, we expect that it would be nondifferential (i.e., not associated with %FGV or salivary hormone levels), and would thus bias the results toward the null. Another limitation is that, despite the fact that the overall sample size in this study was large, the sample size was small for specific subgroup analyses. Studies with larger populations may be better able to detect significant associations between birth characteristics, hormone levels, and breast density where they exist. Selection bias may be present in the sample of patients providing saliva samples for the hormone analyses; women who consented to donate saliva were significantly more likely to be of White Non-Hispanic race and to be of higher socioeconomic status based on education and income, but were not significantly different in terms of family history of breast cancer or current age. This selective participation would only be expected to bias the results if the associations between birth characteristics, hormone levels, and breast density differ by race or socioeconomic status. While we do not anticipate that this would be the case, bias in the results due to selective participation cannot be ruled out. This does, however, limit the ability to generalize the findings here to a broader, more racially diverse population. Finally, the analyses of birth characteristics were intentionally restricted to first births, but it will be important to determine whether the findings for first birth characteristics hold for all births or whether they are unique to the first birth (e.g., whether total duration of breast-feeding has the same association with hormone levels as duration of breast-feeding after the first birth).

5. Summary and conclusions

Expected age and postmenopause related declines in estradiol (E), progesterone (P), dehyroepiandrosterone (DHEA) and testosterone (T) levels were observed. DHEA and T were the only postmenopausal hormone levels significantly associated with any reproductive characteristics: parity and breast feeding for DHEA, age-at-first birth for T. Postmenopausal breast density was borderline significantly negatively

associated with parity, and T was the only hormone level to retain any association with %FGV in multivariable analysis (negative, $p = 0.04$). These findings suggest that first pregnancy effects on later life breast density and cancer risk are not strictly mediated by later life sex steroid hormone levels.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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