Associations of daily 17β-estradiol and progesterone with mammographic density in premenopausal women. The Norwegian EBBA-I Study

Anita Iversen[1], Anne-Sofie Furberg[1,2], Sissi Espetvedt Finstad[3], Anne McTiernan[4], Vidar Flote[3], Ghazwan Alhaidari[3], Giske Ursin[5,6,7], Tom Wilsgaard[1], Peter T. Ellison[8], Grazyna Jasienska[9], Inger Thune[1,3]

[1]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway; [2]Department of Microbiology and Infection Control, University Hospital North Norway, Tromsø, Norway; [3]Department of Oncology, Oslo University Hospital Ullevål, Oslo, Norway; [4]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; [5]Cancer Registry of Norway, Oslo, Norway; [6]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; [7]Department of Preventive Medicine, University of Southern California, Los Angeles, California, USA; [8]Department of Human Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA; [9]Department of Epidemiology and Population Studies, Jagiellonian University Medical College, Krakow, Poland

Abstract

Purpose: To investigate the associations between daily salivary 17β-estradiol and progesterone concentrations and percent mammographic density among premenopausal women enrolled in the Norwegian Energy Balance and Breast cancer Aspects (EBBA)-I Study and followed over the course of an entire menstrual cycle.

Methods: Among 202 healthy women, aged 25-35 years, daily salivary 17β-estradiol and progesterone concentrations were measured. Computer-assisted breast density readings (MADENA) were obtained from digitized mammograms taken between day 7 and 12 of the menstrual cycle. Multivariable linear and logistic regression models examined the associations between ovarian hormones and percent mammographic density.

Results: Compared with women having a low percent mammographic density (< 28.5%), women with a high percent mammographic density (≥ 28.5%) had 25% higher daily 17β-estradiol concentrations (P = 0.007), and 31% higher daily progesterone concentrations (P = 0.010) across the entire menstrual cycle. Compared with women in the first quartile of overall average daily progesterone concentrations, the odds of high percent mammographic density (≥ 28.5%) increased among women in higher progesterone quartiles (Q4 vs. Q1: Odds Ratio 3.70, 95% Confidence interval 1.35-10.11, P_{trend} = 0.011). These associations were even stronger among nulliparous women with an interaction between parity and average daily progesterone in the luteal phase (P = 0.017). We also observed strong associations between serum concentrations of ovarian hormones and percent mammographic density.

Conclusion: Daily 17β-estradiol and progesterone were strongly associated with percent mammographic density in premenopausal women, and could in part explain the association of breast density with increased breast cancer risk.

Introduction

Mammographic density reflects the relative amount of connective and epithelial tissue and fat in the breast, and is a strong and independent risk factor for breast cancer (1-2). Women with the highest percent mammographic density (> 75%) have four to six times greater breast cancer risk compared to women with less dense breasts (3-5). Mammographic density declines at menopause and has been positively associated with both combined estrogen plus progestin use (6-7), and endogenous estrogen levels in postmenopausal women (8). In contrast, knowledge about the association between endogenous ovarian hormones and mammographic density among premenopausal women is limited and inconsistent (9-11).

A woman's lifetime exposure to estrogens has a large cumulative effect on her risk of getting breast cancer (12), and estrogen plays a key role in breast carcinogenesis (13-14). Recently, an association between hormone exposure and mammographic density was observed in women who later developed breast cancer (15). Thus, studies including daily levels of endogenous estrogen and progesterone may be valuable in clinical practice (8). However, other factors may influence mammographic density alone or in combination with ovarian hormones. Parity has been observed to be inversely related to mammographic density (16-19), possibly due to lobular differentiation of breast tissue during pregnancy (19-20).

Previously, in the Norwegian Energy Balance and Breast cancer Aspects (EBBA-I) Study, when analyzing the association between metabolic risk profile and ovarian hormones, we observed a crude positive association between salivary progesterone concentrations and mammographic density using a modified Wolfe's classification (21). Thus, the main aim of the present study was to examine the associations between daily 17β-estradiol and progesterone concentrations across the menstrual cycle among premenopausal women and percent mammographic density assessed by the more accurate computer-assisted method (MADENA) (22).

Materials and methods

Participants and study design

The women participating in the Norwegian EBBA-I Study (2000-2002) were recruited through local media campaigns. A total of 204 women, 25-35 years were included and met the following eligibility criteria; regular menstrual cycles (cycle length: 22-38 days within the previous three

months), no use of any medication, no pregnancy or lactation or use of steroid contraceptives over the previous 6 months, and no gynecological or chronic medical conditions (e.g. diabetes) (21-23). Information including parity and lifestyle factors was collected using questionnaires and interviews. All questionnaires were checked for inconsistencies by one trained nurse. The participants underwent clinical examination at the Clinical Research Center, University Hospital of North Norway (UNN), Tromsø, at three scheduled visits during their menstrual cycle: first visit between day 1 and 5 after onset of the menstrual cycle (early follicular phase), second visit between day 7 and 12 (late follicular phase), and third visit between day 21 and 25 (late luteal phase). Two women were excluded due to missing mammographic data, leaving data from 202 premenopausal women available for the present study.

Daily 17β-estradiol and progesterone

Women collected daily morning saliva samples at home for one entire menstrual cycle, and sampling started on the first day of bleeding (21, 24-25). Hormone assays were run in the Reproductive Ecology Laboratory, Harvard University, Cambridge, USA (25). Saliva samples from 20 consecutive days (reverse cycle days -5 to -24; with -1 implicating the last day of the menstrual cycle) of the women's menstrual cycle was used for 17β-estradiol analysis, and saliva samples from14 consecutive days (reverse cycle days -1 to -14) was used for progesterone analysis (21).

Alignment of the cycles for analysis was based on the identification of the mid-cycle estradiol drop (aligned cycle day 0), which provides a good estimate of the day of ovulation (25). Identification of the drop in salivary 17β-estradiol concentration was not satisfactory for 14 women, and their cycles were not aligned. Overall average salivary 17β-estradiol and progesterone were calculated for all women, and additional indices of average hormone concentrations were calculated for 188 women: "luteal" index (aligned cycle days 0 to +6) and "mid-menstrual" index (aligned cycle days -7 to +6). Maximum peak level corresponds to the highest measured hormone concentration during the aligned cycle (day -1).

Serum samples and clinical examination

Fasting morning blood samples were obtained at all three visits. Fresh serum 17β-estradiol and progesterone concentrations were measured by direct immunometric assay (Immuno-1, Bayer Diagnostics, CITY, Country), sex hormone-binding globulin (SHBG) was measured by an

immunometric method (both Diagnostic Products Corporation (DPC)-Bierman GmbH, Bad Nauheim, Germany), and total cholesterol was determined enzymatically using cholesterol esterase and cholesterol oxidase, and high-density lipoprotein cholesterol (HDL-C) was quantified by direct assay using enzymes modified by polyethylene glycol and dextran sulfate, at the Department of Clinical Chemistry, UNN.

Anthropometric measurements were performed with participants wearing light clothing and no footwear. Height was measured to the nearest 0.5 cm, and weight to the nearest 0.1 kg on an electronic scale. Body mass index (BMI) was calculated in kg/m^2. At the second visit participants underwent a full-body scan to estimate total percent body fat, using dual energy X-ray absorptiometry (DEXA, DPLX-L 2288, Lunar Radiation Corporation, Madison, Wisconsin, USA). Blood pressure was measured (21, 23).

Mammographic density

Bilateral two-view mammograms were obtained at the second visit (day 7-12) using a standard protocol (Furberg 2005). Left craniocaudal mammograms were digitized and imported into a computerized mammographic density assessment program (MADENA) (22). The total breast area was defined on the mammographic image using a special outlining tool. The region of interest (ROI) was then outlined. The mammogram reader used a tinting tool to apply yellow tint to pixels considered to represent areas of mammographic density. The MADENA software estimated the total number of pixels and the number of tinted pixels in the ROI. "Absolute mammographic density" represents the number of the tinted pixels within the ROI, and "percent mammographic density" is the ratio of absolute mammographic density to the total breast area (area of ROI) multiplied by 100. The mammograms were read in four batches, with an equal number of mammograms in each batch. A duplicate reading of 26 randomly selected mammograms from two of the batches showed a Pearson's correlation coefficient of 0.97. The reader was blinded to all characteristics of the study population. The density assessments were conducted by one of the authors (G.U.), and the breast areas were outlined by a research assistant trained by G.U.

Ethical considerations

All the participants signed an informed consent form and the study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

Statistical analysis

Percent mammographic density was dichotomized into high and low density breasts, using the median percent mammographic density (28.5%) as the cut point. Student's t-test, Pearson's chi-squared test or linear regression was used to compare means and proportions of selected characteristics by median split of percent mammographic density (< 28.5%, and ≥ 28.5%). The adjusted odds ratios (ORs) of having above median percent mammographic density (≥ 28.5%) were estimated using logistic regression models. We also stratified the models by parity (parous, nulliparous). Both salivary 17β-estradiol and progesterone concentrations were included as categorical variables (quartiles) in separate analyses. Tests of interaction between parity and hormone concentrations were conducted by including cross product terms in the models. Previous oral contraceptive use was not an independent predictor of percent mammographic density when adjusting for age and BMI, and was not included in the final models. Generalized estimating equation models were used to assess the associations of daily salivary 17β-estradiol and progesterone concentrations with median split of percent mammographic density. Age, BMI, parity and indicator variables of aligned cycle day were included when appropriate as covariates in models. Hormone data were log transformed prior to statistical analyses, but untransformed hormone values are presented in participants' characteristics to facilitate readability. All statistical tests were two-sided using a 5% significance level. Statistical analyses were conducted with STATA version SE 11.0 (Stata Corporation, College Station, Texas, USA).

Results

The participating premenopausal women had a mean age of 30.7 years, mean age at menarche of 13.1 years and none were current users of steroid hormones. The mean (standard deviation) and median (range) percent mammographic density were 29.8% (19.0 %) and 28.5% (15.3 - 78.9%), respectively (data not shown). Nulliparous women had a higher mean percent mammographic density than parous women, 35% versus 25%, respectively ($P < 0.001$, adjusted for age and BMI) (data not shown).

Table 1. Characteristics of the premenopausal women by median split of percent mammographic density. The Norwegian EBBA-I study (n=202)[a]

	Percent mammographic density				
	< 28.5 % (n=101)		≥ 28.5 % (n=101)		*P* value[b]
Age, years	31.5	(31.0,32.1)	29.9	(29.3,30.5)	*<0.001*
Reproductive factor					
Age at menarche, years	12.9	(12.6,13.1)	13.4	(13.1,13.6)	*<0.011*
Cycle length, days	27.9	(27.3,28.5)	28.7	(28.0,29.3)	*0.069*
Number of children	1.35	(1.11,1.59)	0.49	(0.32,0.65)	*<0.001*
Clinical measurements[c]					
Height, cm	167.3	(166.2,168.4)	166.7	(165.3,168.1)	*0.48*
BMI, kg/m^2	26.1	(25.4,26.9)	22.7	(22.1,23.2)	*<0.001*
Total body fat (DEXA), %	37.9	(36.6,39.3)	30.5	(29.2,31.8)	*<0.001*
Systolic blood pressure, mmHg	115.5	(113.0,117.9)	111.0	(109.2,112.9)	*0.004*
Diastolic blood pressure, mmHg	72.1	(70.5,73.7)	69.8	(68.2,71.3)	*0.039*
Daily saliva samples, fasting pmol/l					
Estradiol, overall	17.6	(15.8,19.3)	18.5	(16.8,20.2)	*0.44*
Estradiol, luteal[e]	16.8	(15.0,18.7)	18.3	(16.4,20.2)	*0.28*
Progesterone, overall	119.8	(107.5,132.2)	141.6	(127.3,155.9)	*0.023*
Progesterone, luteal[e]	109.3	(96.5,122.0)	132.4	(117.4,147.3)	*0.020*
Serum samples, fasting					
Total cholesterol[c], mmol/l	4.55	(4.39,4.70)	4.36	(4.20,4.51)	*0.087*
HDL-C[c], mmol/l	1.47	(1.40,1.54)	1.60	(1.54,1.66)	*0.005*
Estradiol, late luteal[d], pmol/l	374.9	(342.6,407.1)	488.0	(445.3,530.7)	*<0.001*
Progesterone, late luteal[d], nmol/l	31.0	(27.6,34.4)	41.0	(36.7,45.2)	*<0.001*
SHBG, late luteal[d], nmol/l	48.9	(44.7,53.2)	56.9	(52.7,61.0)	*0.009*
Oral contraceptive use, years	4.47	(3.74,5.20)	3.22	(2.51,3.94)	*0.017*
Alcohol consumption, units/week	3.11	(2.89,3.33)	3.33	(3.05,3.61)	*0.22*
Energy intake, kJ/day	7943	(7559,8327)	8273	(7907,8638)	*0.22*
Sedentary leisure activity, %		17.8		13.9	*0.44*
Current smokers, %		26.7		17.8	*0.13*

Notes: To obtain conventional unit divide by the following conversion factor: 3.671, estradiol (pg/ml); 3.18, progesterone (ng/ml). Data are expressed as unadjusted mean (95% CI) or percentage.
[b]Student's *t* or chi square test.
[c]Serum samples in early follicular phase: day 1-5 after onset of menstrual cycle, except total tissue fat (DEXA) measured at day 7-12 (late follicular phase) after onset of the menstrual cycle.
[d]Serum samples in late luteal phase: day 21-25 after onset of menstrual cycle.
[e]Daily saliva samples in luteal phase: aligned cycle day 0, +6 (n = 188).
Abbreviations: CI, confidence interval; BMI, body mass index; DEXA, dual-energy x-ray absorptiometry; HDL-C, high-density lipoprotein cholesterol; SHBG, sex hormone-binding globulin

In the present study, younger age (P < 0.001), older age at menarche (P = 0.011), and lower parity (P < 0.001) were positively associated with percent mammographic density (Table 1). Furthermore, BMI and body fat (% DEXA) were inversely related to percent mammographic density (all P < 0.001). Unadjusted serum concentrations of estradiol (P < 0.001), progesterone (P < 0.001), and SHBG (P < 0.009) in the luteal phase were positively associated with percent mammographic

density (Table 1). The association with serum SHBG disappeared after adjustment for age and BMI (P = 0.52) (data not shown). Higher unadjusted salivary concentrations of overall average and luteal phase progesterone were positively associated with percent mammographic density (P = 0.023 and P = 0.020, respectively) (Table 1).

17β-estradiol concentration and percent mammographic density

When comparing premenopausal women with high (≥ 28.5%) versus low (< 28.5%) percent mammographic density, we observed 25% higher daily salivary 17β-estradiol concentrations in the mid-menstrual phase (P = 0.007), 23% higher daily salivary 17β-estradiol concentrations in the luteal phase (P = 0.024), and a 21% higher maximum peak salivary 17β-estradiol concentration (P = 0.031), all adjusted for age and BMI (Table 2 and Figure 1A). Moreover, women in the higher quartiles of overall average salivary 17β-estradiol concentrations had higher adjusted odds of high percent mammographic density (≥ 28.5%) compared to women in the lower quartile (Q4 vs.Q1: OR 2.69, 95% CI 0.97-7.51, P_{trend} = 0.031) (Table 3 and Figure 1A). A similar relationship was observed in the luteal phase (Q4 vs. Q1: OR 2.58, 95% CI 0.91-7.33, P_{trend} = 0.058) (Table 3 and Figure 1A). Serum estradiol concentrations in the late follicular phase and the late luteal phase were positively associated with percent mammographic density (P = 0.050 and P = 0.006, respectively) (Table 2).

When stratifying by parity, high (≥ 28.5%) versus low (< 28.5%) percent mammographic density was associated with 35% higher daily salivary 17β-estradiol concentrations in the mid-menstrual phase among nulliparous women (P = 0.011) (Figure 1C). Nulliparous women in the higher quartiles of salivary 17β-estradiol concentrations in the luteal phase, had higher adjusted odds of high percent mammographic density (≥ 28.5%) compared to nulliparous women in the lower quartile (P_{trend} = 0.010) (data not shown). A similar relationship was observed for overall average salivary17β-estradiol concentrations among nulliparous women (P_{trend} = 0.063) (data not shown). No association between salivary 17β-estradiol concentration and percent mammographic density was observed among parous women (Figure 1C). No interaction between salivary 17β-estradiol concentration and parity was observed.

Table 2. Age and BMI adjusted means of 17β-estradiol and progesterone concentrations in saliva and serum for the premenopausal women by median split of mammographic density*. The EBBA-I Study (n = 202)

	Percent mammographic density		
	< 28.5 % (n=101)	≥ 28.5 % (n=101)	*P* value[a]
Estradiol, pmol/l			
Daily saliva samples			
Overall	12.70 (11.41,14.13)	15.65 (14.06,17.41)	*0.012*
Mid-menstrual[b]	13.29 (11.94,14.80)	16.91 (15.13,18.90)	*0.007*
Luteal[c]	12.65 (11.26,14.21)	15.52 (13.76,17.50)	*0.024*
Maximum peak[d]	24.43 (21.83,27.35)	29.63 (26.38,33.27)	*0.031*
Serum samples			
Early follicular[e]	147.6 (135.4,159.9)	143.6 (131.4,155.9)	*0.68*
Late follicular[f]	371.4 (315.6,429.5)	461.1 (401.3,523.4)	*0.050*
Late luteal[g]	378.9 (342.3,416.5)	459.9 (422.1,499.8)	*0.006*
Progesterone			
Daily saliva samples, pmol/l			
Overall	84.59 (75.15,95.21)	102.89 (91.41,115,80)	*0.033*
Luteal[c]	81.02 (71.03,92.41)	111.66 (97.45,127.94)	*0.010*
Serum samples, nmol/l			
Luteal[g]	32.2 (28.1,36.3)	39.7 (35.6,43.9)	*0.020*

Notes: To obtain conventional unit divide by the following conversion factor: 3.671, estradiol (pg/ml); 3.18, progesterone (ng/ml).
*Reported as arithmetic mean for serum progesterone and geometric means for all other samples.
[a]Linear regression and generalized estimating equation.
[b]Daily saliva samples in mid-menstrual phase: aligned cycle day -7,+6 (n = 188).
[c]Daily saliva samples in luteal phase: aligned cycle day 0,+6 (n = 188).
[d]Maximum peak:aligned cycle day -1 (n = 188).
[e]Serum samples in early follicular phase: day 1-5 after onset of menstrual cycle.
[f]Serum samples in late follicular phase: day 7-12 after onset of menstrual cycle.
[g]Serum samples in late luteal phase: day 21-25 after onset of menstrual cycle.
Abbreviations: CI, confidence interval; BMI, body mass index.

Progesterone concentration and percent mammographic density

When comparing premenopausal women with high (≥ 28.5%) versus low (< 28.5%) percent mammographic density, we observed 31% higher daily salivary progesterone concentrations in the luteal phase of the menstrual cycle, adjusted for age and BMI (P = 0.010) (Table 2 and Figure 1B). Women in the higher quartiles of overall average salivary progesterone concentrations had higher adjusted odds of high percent mammographic density (≥ 28.5%) compared to women in the lower quartile (Q4 vs. Q1: OR 3.70, 95% CI 1.35-10.11, P_{trend} = 0.011) (Table 3 and Figure 1B). A similar association was observed in the luteal phase with highest adjusted odds for mammographic density

≥ 28.5% observed in the third progesterone quartile (Q3 vs. Q1: OR 4.92, 95 % CI 1.67-14.44, P_{trend} = 0.032), (Table 3 and Figure 1B). Serum progesterone concentration in the luteal phase was positively associated with mammographic density (≥ 28.5%) (P = 0.020), (Table 2).

When stratifying by parity, high (≥ 28.5%) versus low (< 28.5%) percent mammographic density was associated with 36% higher daily salivary progesterone concentrations across the entire luteal phase among nulliparous women (P = 0.03) (Figure 1D). Furthermore, nulliparous women in the higher quartiles of overall average salivary progesterone concentrations, had higher adjusted odds of high percent mammographic density (≥ 28.5%) compared to nulliparous women in the lower quartile (P_{trend} = 0.028) (data not shown). A similar association was observed in the luteal phase among nulliparous women (P_{trend} = 0.005) with highest adjusted odds for mammographic density ≥ 28.5% observed in the third progesterone quartile (data not shown). There was an interaction between parity and luteal phase concentrations of salivary progesterone (P = 0.017).

Table 3. Odds Ratios (95% CI) for high percent mammographic density (≥ 28.5 %) by quartiles of daily salivary 17β-estradiol and progesterone concentrations among the premenopausal women. The EBBA-I Study (n = 202)

	Q1	Q2	Q3	Q4	P_{trend}[a]
17β-estradiol, pmol/l					
Overall	1.0 (ref)	1.38 (0.54-3.55)	2.37 (0.87-6.44)	2.69 (0.97-7.51)	*0.031*
Luteal[b]	1.0 (ref)	1.43 (0.53-3.89)	2.00 (0.72-5.51)	2.58 (0.91-7.33)	*0.058*
Progesterone, pmol/l					
Overall	1.0 (ref)	2.35 (0.88-6.28)	2.99 (1.07-8.34)	3.70 (1.35-10.11)	*0.011*
Luteal[b]	1.0 (ref)	3.63 (1.24-10.61)	4.92 (1.67-14.44)	3.04 (1.06-8.74)	*0.032*

[a]Logistic regression, adjusted for age, body mass index, and parity (parous, nulliparous)
[b]Daily saliva samples in luteal phase: aligned cycle day 0, +6, (n = 188).
Abbreviations: CI, confidence interval.

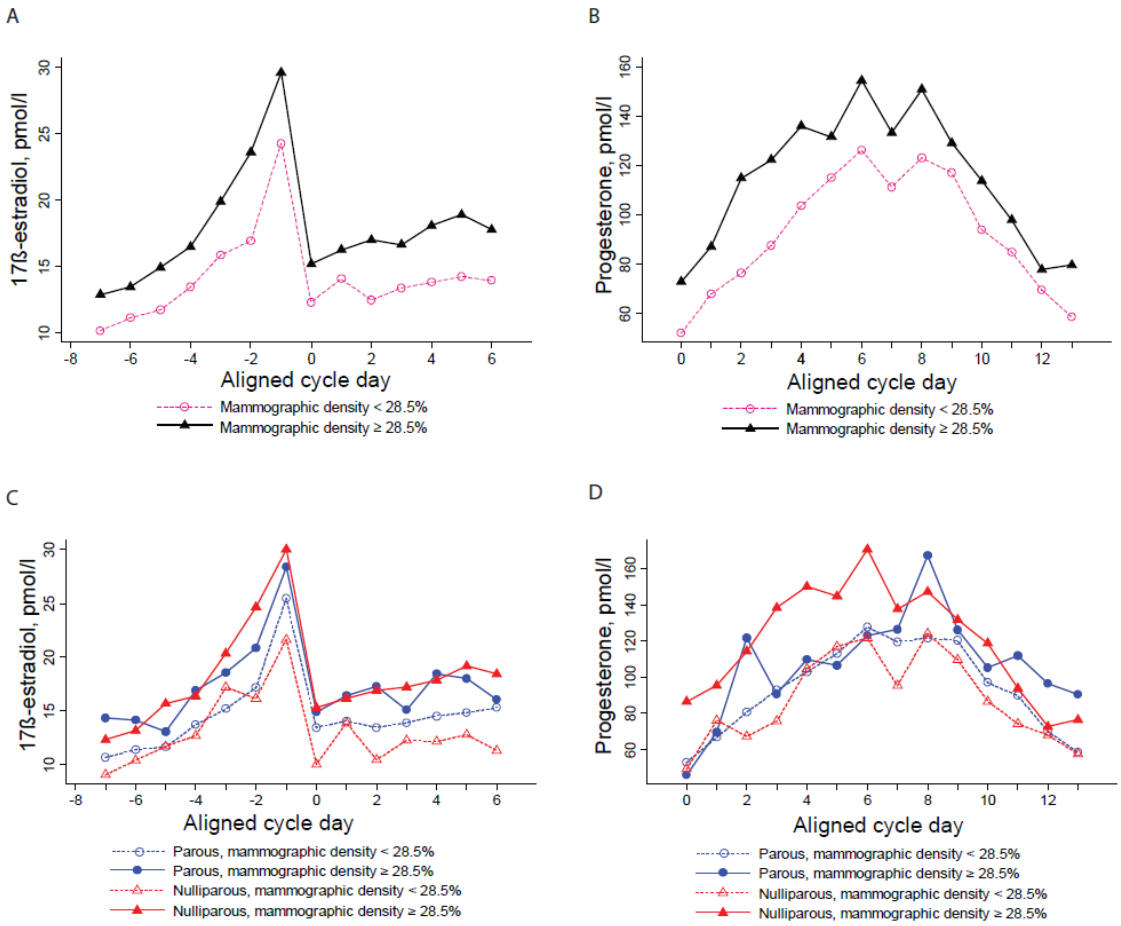

Figure 1. Age and body mass index adjusted daily salivary concentration (geometric means) of A) C) 17β-estradiol (aligned cycle day -7, +6), and B) D) progesterone (aligned cycle day 0, +13) for A) B) premenopausal women categorized by median split of percent mammographic density; < 28.5 % (n=96), ≥ 28.5 % (n=92) and C) D) premenopausal women categorized according to parity and percent mammographic density (median split); nulliparous and percent mammographic density < 28.5 % (n=31), nulliparous and percent mammographic density ≥ 28.5 % (n=66), parous and percent mammographic density < 28.5 % (n=65), parous and percent mammographic density ≥ 28.5 % (n=26). The EBBA-I Study.

Discussion

Our finding of a strong positive association between daily endogenous estrogen and progesterone concentrations and percent mammographic density in premenopausal women extends previous studies. Most of the previous studies have focused on the relationship between sex steroids and mammographic density among postmenopausal women, while studies examining the associations between endogenous ovarian hormone profiles over the entire menstrual cycle and mammographic density among premenopausal women have been sparse. However, our observation that women with a high percent mammographic density (≥ 28.5%) had 25% higher daily 17β-estradiol

concentrations, and 31% higher daily progesterone concentrations, across the entire menstrual cycle compared with women having a low percent mammographic density (< 28.5%) is partly supported. Interestingly, a positive association between follicular phase serum estradiol concentrations and percent mammographic density among women was observed (mean age: 42.4 years) (11), and serum concentration of progesterone was positively associated with percent mammographic density among premenopausal women (10). Furthermore, total urinary estrogen metabolites were positively associated with percent mammographic density in premenopausal women (26), and a direct association was observed between preovulatory and luteal phase urinary estrone glucuronide and percent mammographic density (27). In contrast to our results, the magnitude of the association was reduced after adjustment for BMI, but these women were mostly parous with mean age 48.4 years (27). In addition, urinary estrone glucuronide may reflect different biological pathways and mechanisms than serum and salivary estradiol.

The suggested effect of both endogenous estrogen and progesterone on mammographic density in premenopausal women in our study is supported by reports from randomized trials including postmenopausal women, showing that combined estrogen plus progesterone use is associated with larger changes in percent mammographic density than estrogen use alone (6-7, 14). These data also hypothesize that progesterone may be an even stronger predictor of mammographic density than estrogen (6-7, 14, 28). So far, reports from observational studies among postmenopausal women are conflicting (8), however, in some studies, mammographic density increased with higher endogenous estrogen (29-30) and progesterone concentrations (29).

Furthermore, our results are consistent with the hypothesis that a positive association between circulating free estrogen and progesterone and breast cancer risk may be mediated, in part, by increasing mammographic density. Recently, changes in mammographic density by hormone exposure were observed to be stronger in women who later developed breast cancer (15). Whether mammographic density or a specific threshold of mammographic density in early adulthood is predictive of breast cancer risk later in life is not known. Breast tumors have been shown to arise predominantly within the radiodense areas of the breast (31). Thus, mammographic density and levels of endogenous estradiol and progesterone at a given age may in combination be important markers for breast cancer risk later in life. Our findings support the hypothesis that lowering levels

of estradiol and progesterone (32-35) in young women may reduce mammographic density and improve diagnostics and breast cancer risk assessment (36-37).

We observed that the associations between ovarian hormones and breast density were stronger among nulliparous women than among parous women, suggesting that the relationship between ovarian hormones and breast density may vary by parity among premenopausal women. Such an interaction may be explained by the fact that percent mammographic density decreases after first full-term pregnancy (19). Parity-induced molecular changes in growth factors, cell fate, p53 activation or induction of a specific genomic signature in the breast may be involved (38-39). Thus, we hypothesize that relative to the breast tissue of parous women, the breast tissue of nulliparous women may be more susceptible to higher endogenous ovarian hormone concentrations influencing percent mammographic density. Also, others have suggested that any effect of estrogen and progesterone on breast tissue may vary due to a woman's reproductive status (40) of importance for breast carcinogenesis (14, 41-42). Interestingly, single nucleotide polymorphisms on genes involved in the estrogen pathway have been associated with mammographic density among premenopausal nulliparous women only (43).

Our results are strengthened by the collection of daily salivary measurements of unbound estradiol and progesterone concentrations across an entire menstrual cycle (25, 44-45), following strict procedures and validated methods (21, 24-25). Mammographic density was assessed within a narrow time frame in late follicular phase (between days 7-12) (46). The computer-assisted method used to quantify mammographic density has been shown to give a superior prediction of breast cancer risk compared with qualitative methods (2, 4). All mammograms were read by one experienced blinded reader, and the assessed mammographic density was negatively associated with age, BMI and parity (47-48). However, the small sample size in the present study and few previous reports underline the need for further studies.

In summary, the present findings support the hypothesis that both daily endogenous estradiol and progesterone concentrations are strongly associated with percent mammographic density in premenopausal women in a dose-response manner. Furthermore, our results suggest that nulliparous premenopausal women may be more susceptible to ovarian hormones in prediction of

mammographic density. Interventions to lower estradiol and progesterone in premenopausal women, such as aerobic physical activity and low-fat diets, could aid in reducing density thereby reducing breast cancer risk and improving sensitivity of premenopausal mammogram screening. However, more studies including ovarian hormones across the menstrual cycle are needed to confirm and improve the clinical implications of these findings.

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