# Premenopausal Mammographic Density in Relation to Cyclic Variations in Endogenous Sex Hormone Levels, Prolactin, and Insulin-like Growth Factors

Kate Walker,[1] Olivia Fletcher,[2] Nichola Johnson,[2] Ben Coupland,[2] Valerie A. McCormack,[1] Elizabeth Folkerd,[3] Lorna Gibson,[1] Stephen G. Hillier,[4] Jeff M. Holly,[5] Sue Moss,[6] Mitchell Dowsett,[3] Julian Peto,[1,7] and Isabel dos Santos Silva[1]

[1]Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; [2]Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research; [3]The Academic Department of Biochemistry, The Royal Marsden Hospital, London, United Kingdom; [4]Centre for Reproductive Biology, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, United Kingdom; [5]University Department of Clinical Science at North Bristol, Southmead Hospital, Bristol, United Kingdom; and [6]Cancer Screening Evaluation Unit and [7]Cancer Research UK Epidemiology and Genetics Group, The Institute of Cancer Research, Sutton, Surrey, United Kingdom

## Abstract

**Mammographic density is strongly associated with breast cancer risk, and endogenous hormones, which are risk factors for breast cancer, may be involved in the mechanism. This cross-sectional study of 494 premenopausal women is the first to account for cyclic variations in estrogen levels, by measuring urinary estrone glucuronide (E1G) in the periovulatory and luteal phases of the menstrual cycle, and to assess the role of androgens. Computer-assisted density readings were obtained from digitized mammograms. Mean ovulatory E1G level and daily E1G load were both positively associated with percent density before adjustment for body mass index (BMI), with women in the top fourth having 10.2% (95% CI: 2.9%, 18.1%) and 8.9% (1.7%, 16.7%), respectively, higher density than those in the bottom fourth ($P_{trend}$ before/after BMI adjustment = 0.006/0.11 and 0.01/0.13, respectively). Neither the peak nor luteal E1G levels were predictive of density after adjustment for E1G levels at other points in the cycle. The plasma androgens testosterone, androstenedione, and dehydroepiandrosterone sulfate were negatively associated with density. In mutually adjusted analyses, density was positively associated with insulin-like growth factor (IGF)-I and negatively with IGF-II ($P_{trend}$ = 0.006 for both) but not with IGF binding protein-3. There was also weak evidence of a positive association of prolactin with density. The study supports the hypothesis that endogenous hormones affect density in premenopausal women; in particular, it shows a positive association between estrogen levels and density and suggests that the mean level throughout the cycle is the most biologically relevant measure. Most of these hormone-density associations were attenuated with further adjustment for BMI.** [Cancer Res 2009;69(16):6490–99]

## Introduction

Mammographic density is an established risk factor for breast cancer, with women whose breast density is at least 75% having four times the risk of developing breast cancer than those whose breast density is very low (1). The biological mechanisms by which density is associated with breast cancer risk are unclear. Cross-sectional studies have attempted to relate breast density with exposure to mitogens known to be associated with breast cancer risk, such as sex steroid hormones, prolactin, and insulin-like growth factors (IGF), but these were conducted more extensively in postmenopausal women (2). The role of sex steroid hormones on mammographic density in premenopausal women was examined in only two studies (3, 4), with none having attempted to measure estrogen levels throughout the menstrual cycle or having assessed the role of androgens. Few studies have examined the role of prolactin (4, 5) or IGFs (4–10) on mammographic density in premenopausal women, and they tended to be fairly small, typically <300 subjects.

In this study, we examined mammographic density in relation to several sex steroid hormones, prolactin, and IGFs among premenopausal women. This study is the first to have collected serial estrogen measurements throughout the woman's menstrual cycle and to have examined the role of androgens on density in premenopausal women.

## Materials and Methods

**Subjects.** The Mammography, Oestrogens and Growth factors study is an observational study nested within a trial of annual mammographic screening in young women conducted in Britain (11). About 54,000 women ages 39 to 41 years were randomized to the intervention arm in 1991 to 1997 and offered annual mammograms until age 48 years. In 2000 to 2003, women in the intervention arm who were still participating in this trial were invited to participate in the Mammography, Oestrogens and Growth factors study by providing a blood sample and completing a brief questionnaire about their reproductive and lifestyle characteristics, including self-reported height and weight. Over 8,000 women were enrolled. For the present study, all 800 Caucasian women from the Mammography, Oestrogens and Growth factors study who had never been diagnosed with cancer, were still having regular menstrual cycles (between 21 and 35 days), and were not on hormone replacement therapy or oral contraceptives were invited to participate by providing repeat urine samples throughout the menstrual cycle.

Urinary estrone glucuronide (E1G) is a principal metabolite of the serum estrogens and, when measured in early morning urine samples, is highly

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

**Requests for reprints:** Isabel dos Santos Silva, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, The Institute of Cancer Research, Keppel Street, London WC1E 7HT, United Kingdom. Phone: 44-20-7927-2113; Fax: 44-20-7436-4230; E-mail: isabel.silva@lshtm.ac.uk.



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correlated with the serum estradiol curve, with a delay of 1 to 2 days (12). A protocol was developed for capturing peak follicular phase E1G levels as well as luteal phase E1G and pregnanediol glucuronide (PG) levels by asking women to provide a set of early morning urine samples on 6 consecutive days estimated to include their day of ovulation plus one sample in their luteal phase. Their date of ovulation was predicted to be 14 days before the date of their next cycle, and the latter was estimated from the date of the first day of their last menstrual period and their reported usual cycle length (Fig. 1). Women were sent a pack containing material for collecting and returning the samples by post to our laboratory, instructions for counting from day 1 (onset of menses) to the first sampling day, and forms for recording the date of each sample. Sampling days were the 3 days immediately preceding the predicted day of ovulation, the predicted ovulation day, and the first, second, and seventh days after predicted ovulation. E1G and PG measurements were adjusted for urinary creatinine levels, as this can be used as a measure of urine concentration, providing circulating levels of creatinine are in the normal range. Thus, samples where the within-woman (12 observations on 9 women) or between-woman (2 women) residual log-transformed creatinine level was beyond $\pm 3$ SDs were excluded. Sets of urine samples were considered to be complete if they included at least four samples on days estimated to be close to ovulation plus one sample estimated to be in the luteal phase of their cycle. A luteal PG/creatinine level $\geq$0.3 mmol/mol was used as an indicator that the woman had ovulated.

In all, 533 women provided repeat urine samples; for 494, mammograms could be retrieved from UK screening centers. Of these, 484 (98%) provided a blood sample and were included in the plasma analyses; 342 (69%) provided a complete set of urine samples and had a luteal PG/creatinine $\geq$0.3 mmol/mol; these were included in the urinary analyses (of the 152 excluded, 18% provided incomplete sets and 71% had a luteal PG/creatinine <0.3 mmol/mol).

**Mammographic measurements.** The mammograms taken closest to the time of urine collection were selected for these analyses. Mediolateral oblique or, if these were not available (1% of women), craniocaudal mammograms for both right and left breasts were digitized using an Array 2905 laser digitizer with optical density range 0 to 4.0, 12-bit depth, and pixel size of 50 μm (Array Corporation Europe). Density readings were done by a single trained reader (I.S.S.) using the interactive threshold method as implemented by the Cumulus software (13). The user's choice of grayscale thresholds defined the edge of the breast and the edge of the dense area within the breast, with estimation of total breast area, areas of dense (fibroepithelial) and lucent (fatty) tissues, and percent density (100 × absolute dense area/total breast area) being fully automated. Films were first stripped of personal identifiers and then read

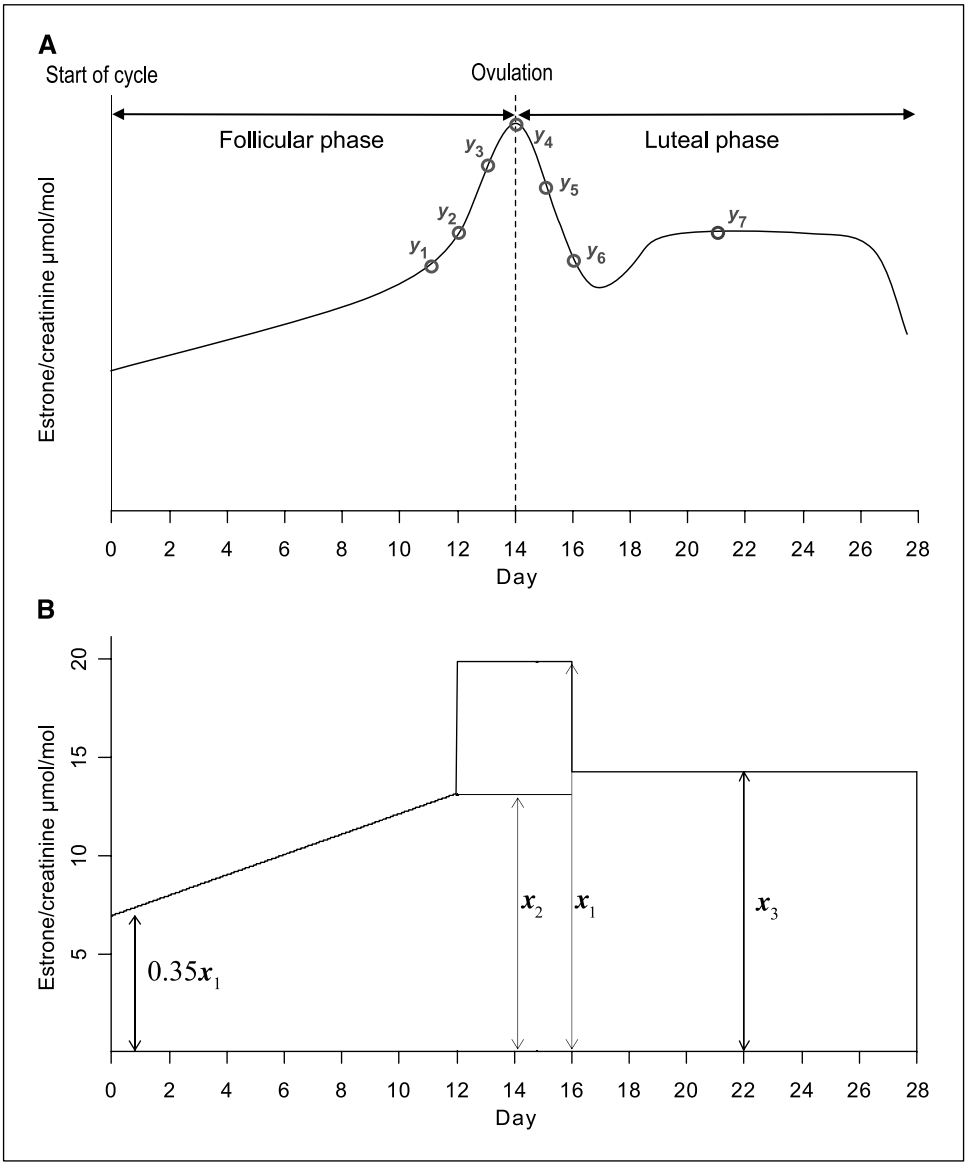


**Figure 1.** *A,* schematic representation of levels of urinary E1G measurements throughout the menstrual cycle and the timing of sample collection. *B,* $x_1$ = maximum three-point moving average of $y1$ to $y6$ [geometric mean and 95% CI, 19.5 (18.7,20.4) μmol/mol]; $x_2$ = mean of $y1$ to $y6$, excluding those points in three-point moving average, $x1$ [geometric mean and 95% CI, 13.1 (12.6,13.6) μmol/mol]; $x_3$ = luteal measurement, $y_7$ [geometric mean and 95% CI, 14.2 (13.5,14.8) μmol/mol].

**Table 1.** Baseline, hormonal, and mammographic characteristics of the study subjects

| Participant characteristics | *n* | Mean (SD) |
|---|---|---|
| Age at mammography (y) | 493 | 48.4 (1.2) |
| Height (cm) | 492 | 163 (6) |
| Weight (kg)* | 489 | 64.9 (14) |
| BMI (kg/cm$^2$)* | 488 | 24.7 (5.3) |
| Age at first full-term pregnancy (y) | 426 | 25.8 (5.2) |
| | | *n* (%) |
| No. full-term pregnancies | 494 | |
| 0 | | 67 (14) |
| 1-2 | | 298 (60) |
| ≥3 | | 129 (26) |
| Current smoker | 490 | 58 (12) |
| **Hormones and growth factors** | ***n*** | **Median (25th-75th percentile)** |
| Mean E1G† (μmol/mol) | 342 | 16.4 (13.3-21.0) |
| Peak E1G† (μmol/mol) | 342 | 19.7 (15.7-25.1) |
| Luteal E1G† (μmol/mol) | 342 | 14.3 (10.9-18.8) |
| PG† (mmol/mol) | 458 | 0.6 (0.31-0.92) |
| IGF-I (ng/mL) | 484 | 144 (121-172.1) |
| IGF-II (ng/mL) | 484 | 842 (695-997) |
| IGFBP-3 (ng/mL) | 484 | 4,319 (3,762-4,828) |
| Testosterone (nmol/L) | 382 | 1.08 (0.62-1.85) |
| Androstenedione (nmol/L) | 382 | 4.6 (3.6-5.8) |
| DHEA (nmol/L) | 382 | 14.7 (10.5-20.0) |
| Dehydroepiandrosterone sulfate (DHEAS; μmol/L) | 382 | 2.66 (1.89-3.57) |
| Prolactin (mIU/L) | 449 | 265 (188-368) |
| SHBG (nmol/L) | 382 | 52.4 (39.5-69.5) |
| **Mammographic density** | ***n*** | **Median (25th-75th percentile)** |
| Dense area (cm$^2$) | 494 | 35.3 (18.4-51.7) |
| Lucent area (cm$^2$) | 494 | 92.1 (59.6-134.7) |
| Total breast area (cm$^2$) | 494 | 130.8 (103.6-171.3) |
| Percent density (%) | 494 | 27.8 (13.7-44.1) |
| **Years between mammography and** | | ***n*** **(%)** |
| Urine collection | | |
| <1 | 342 | 251 (73) |
| 1-2 | | 89 (26) |
| 3-4 | | 1 (0.3) |
| >5 | | 1 (0.3) |
| Blood collection | | |
| <1 | 484 | 256 (53) |
| 1-2 | | 217 (45) |
| 3-4 | | 9 (2) |
| >5 | | 2 (0.4) |

*Median and interquartile range instead of mean and SD.
† Adjusted for creatinine concentration.

in a random order without knowledge of any characteristics of the subjects. A 10% random sample was read twice independently by the same observer [intraclass correlation coefficient, 0.91 (95% CI: 0.87, 0.94)].

**Laboratory measurements.** Laboratory assays were conducted blindly in relation to the questionnaire and mammographic data. If there was a limited amount of plasma available, the assays were prioritized in the following order: IGFs, prolactin, androgens, and sex hormone binding globulin (SHBG). Plasma levels of sex hormones and IGFs were analyzed using commercially available radioimmunoassay kits (see Supplementary information), except IGF binding protein-3 (IGFBP-3). Urinary levels of E1G and PG were measured by ELISA (14).

**Statistical analysis.** Several measures were used to summarize a woman's E1G curve. The mean ovulatory E1G level was estimated as the mean of all follicular measurements obtained around the ovulation peak,

labeled $y_1$ to $y_6$ in Fig. 1*A*. The maximum of a three-point moving average of the follicular measures, $y_1$ to $y_6$, was used as an estimate of the peak follicular E1G level. The single measurement obtained in the luteal phase, $y_7$, was used to estimate the luteal plateau. An estimate of the area under the E1G curve (AUC) for the whole cycle was obtained by approximating the shape of the curve with simple geometric shapes as shown in Fig. 1*B*. The height at each point of the curve was estimated by a summary measure of the E1G measurements, $y_1$ to $y_7$, as detailed in Fig. 1*B*. The ratio of the

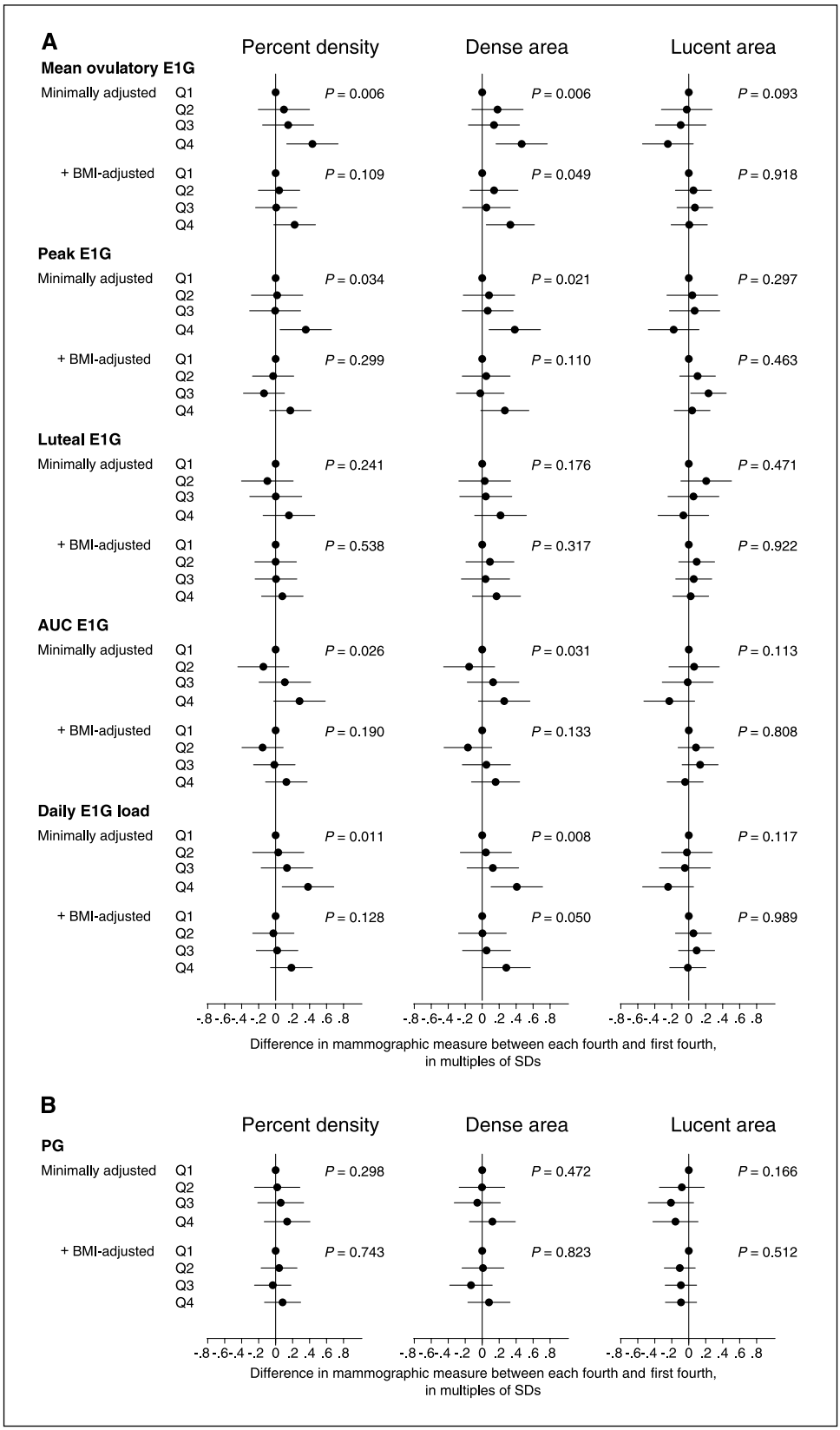


**Figure 2.** Mammographic density in relation to (*A*) urinary E1G levels throughout the menstrual cycle and (*B*) urinary PG levels in the luteal phase. Mean ovulatory E1G = mean of $y_1$ to $y_6$. Peak E1G = $x_1$. Luteal E1G = $y_7$. AUC E1G = area of geometric approximation to curve in Fig. 1. Daily E1G load = AUC E1G/cycle length.

height of the E1G curve at the start of a women's cycle relative to her peak level was estimated from the curve produced by Meyer and colleagues, based on daily serum estradiol measurements from 674 women, to be 0.35 (15). Each woman's daily exposure to estrogen was calculated by dividing her AUC by her self-reported menstrual cycle length. The molar ratios of IGF-I and IGF-II to that of IGFBP-3 were calculated by first converting IGFs and IGFBP-3 from ng/mL to nmol/L by multiplying their values by 0.13 and 0.025, respectively. All assay values were above the lower detection limit, except 4% of testosterone measurements, which were assigned the value of this limit (0.14 nmol/L) in the analysis.

The mammographic measures assigned to each woman were the mean of the values from her left and right mammograms. Square-root transformations were made on each of percent density, dense area ($cm^2$), and lucent area ($cm^2$) to achieve normality of the residuals in the regression models. Linear regression models were fitted to each of the transformed mammographic measures on the fourths of each hormone's distribution to provide mean differences (and 95% CI) in mammographic measures between each fourth (Q1-Q4) and the bottom one (Q1, reference category). Models were fitted to provide three estimates of these mean differences: (*a*) minimally adjusted, which were adjusted for mammographic view, age at mammography, time from sample collection to mammography, sample storage time, and laboratory assay batch; (*b*) body mass index (BMI) adjusted, which were additionally adjusted for BMI; and (*c*) fully adjusted, which were further adjusted for age at first full-term pregnancy, number of full-term pregnancies, and current smoking status. These estimates are presented in multiples of the SD. A linear relationship was assumed between the transformed mammographic measures and each potential confounder, except that BMI was assumed to be quadratic, and current smoking status was dichotomous. Two-sided *P* values of the linear trend ($P_{trend}$) on the fourths were obtained. To facilitate interpretation, mean differences in mammographic measures between fourths were back-transformed to their original measurement scales for reference values of 30% for percent density, 35 $cm^2$ for dense area, and 90 $cm^2$ for lucent area (corresponding to the median values in the study population).

## Results

### Study Population

The characteristics of the 494 participants are summarized in Table 1. There were no differences in baseline characteristics between these women and those for whom mammograms were not available or between the 342 women included in the urinary analyses and those who were not eligible. Mammograms were available within 1 year of collection of the urine samples for 73% of women and within 3 years for 99%. Blood samples were taken within 1 year of mammography for 53% of participants and within 3 years for 98%. Hormone levels were uncorrelated with storage time.

As expected, BMI was strongly inversely correlated with both percent density and area of dense tissue and positively associated with lucent area ($P_{trend}$ < 0.001 for all). Percent density was four times (ratio, 0.24; 95% CI, 0.20, 0.29) lower in women with a BMI > 28 $kg/m^2$ compared with women with a BMI < 22 $kg/m^2$ (Supplementary Table S1). Several hormones were correlated, in fully adjusted analyses, with BMI; in particular, there were positive associations with testosterone and negative associations with androstenedione and SHBG. Other subjects' characteristics were also weakly associated with mammographic measures and/or hormone levels (Supplementary Table S1).

### Main Findings

Overall, the findings showed that percent density was positively associated with most E1G measures but negatively associated with androgen levels. Mutually adjusted analyses also showed a positive association of percent density with IGF-I and an inverse association with IGF-II but no association with IGFBP-3. Further adjustment for BMI attenuated the magnitude of the hormone-density associations and removed the effect of IGF-I and IGF-II. Associations of hormone levels with dense area were in the same direction as those with percent density. Associations with lucent area reflected essentially the strong correlation between this mammographic measure and BMI; hence, they were removed on adjustment for this variable.

**Table 2.** Adjusted mean differences* (95% CI) in percent density across fourths of selected hormone levels for a reference value of 30%

| | Percent density | |
|---|---|---|
| | Minimally adjusted | +BMI-adjusted |
| Mean ovulatory E1G | | |
| Q2 | 2.16 (−4.27, 9.30) | 0.89 (−4.26, 6.50) |
| Q3 | 3.29 (−3.24, 10.53) | 0.14 (−4.92, 5.66) |
| Q4 | 10.15 (2.91, 18.11) | 5.05 (−0.48, 11.05) |
| Daily E1G load | | |
| Q2 | 0.68 (−5.59, 7.66) | −0.58 (−5.58, 4.88) |
| Q3 | 2.97 (−3.59, 10.25) | 0.38 (−4.74, 5.97) |
| Q4 | 8.86 (1.71, 16.73) | 4.17 (−1.29, 10.12) |
| PG | | |
| Q2 | 1.05 (−4.58, 7.25) | 2.06 (−2.52, 6.99) |
| Q3 | 2.16 (−3.56, 8.45) | 0.12 (−4.32, 4.92) |
| Q4 | 3.56 (−2.32, 10.02) | 2.61 (−2.02, 7.60) |
| Testosterone | | |
| Q2 | 1.73 (−4.24, 8.33) | 2.81 (−2.30, 8.36) |
| Q3 | −4.87 (−10.14, 1.01) | −2.09 (−6.77, 3.01) |
| Q4 | −7.79 (−12.75, −2.20) | −0.88 (−5.78, 4.48) |
| Dehydroepiandrosterone sulfate | | |
| Q2 | 2.28 (−3.81, 9.00) | 1.10 (−3.87, 6.50) |
| Q3 | −3.63 (−9.05, 2.40) | −2.56 (−7.17, 2.46) |
| Q4 | −5.71 (−11.02, 0.25) | −2.71 (−7.43, 2.46) |
| SHBG | | |
| Q2 | 6.32 (0.00, 13.25) | 0.78 (−4.28, 6.30) |
| Q3 | 13.32 (6.48, 20.75) | 3.33 (−2.02, 9.15) |
| Q4 | 19.25 (11.93, 27.17) | 4.53 (−1.11, 10.67) |
| Prolactin | | |
| Q2 | 0.14 (−5.29, 6.11) | −1.22 (−5.51, 3.41) |
| Q3 | 0.05 (−5.38, 6.01) | 1.22 (−3.27, 6.04) |
| Q4 | 3.10 (−2.63, 9.38) | 2.93 (−1.69, 7.90) |
| | IGF-adjusted | +BMI-adjusted |
| IGF-I | | |
| Q2 | 1.20 (−4.29, 7.22) | −0.37 (−4.78, 4.41) |
| Q3 | 3.90 (−2.07, 10.45) | 1.25 (−3.50, 6.40) |
| Q4 | 9.88 (2.91, 17.52) | 1.67 (−3.53, 7.34) |
| IGF-II | | |
| Q2 | −3.09 (−8.37, 2.76) | −2.11 (−6.54, 2.70) |
| Q3 | −8.08 (−13.08, −2.44) | −2.98 (−7.62, 2.10) |
| Q4 | −9.82 (−15.23, −3.57) | −2.64 (−7.97, 3.26) |
| IGFBP-3 | | |
| Q2 | 4.03 (−2.03, 10.67) | 2.67 (−2.19, 7.93) |
| Q3 | −0.70 (−7.02, 6.39) | −0.22 (−5.47, 5.54) |
| Q4 | −3.78 (−10.54, 3.97) | −1.14 (−7.01, 5.40) |

*Reference category: bottom fourth, Q1, adjusted mean differences in mammographic measures = 0.

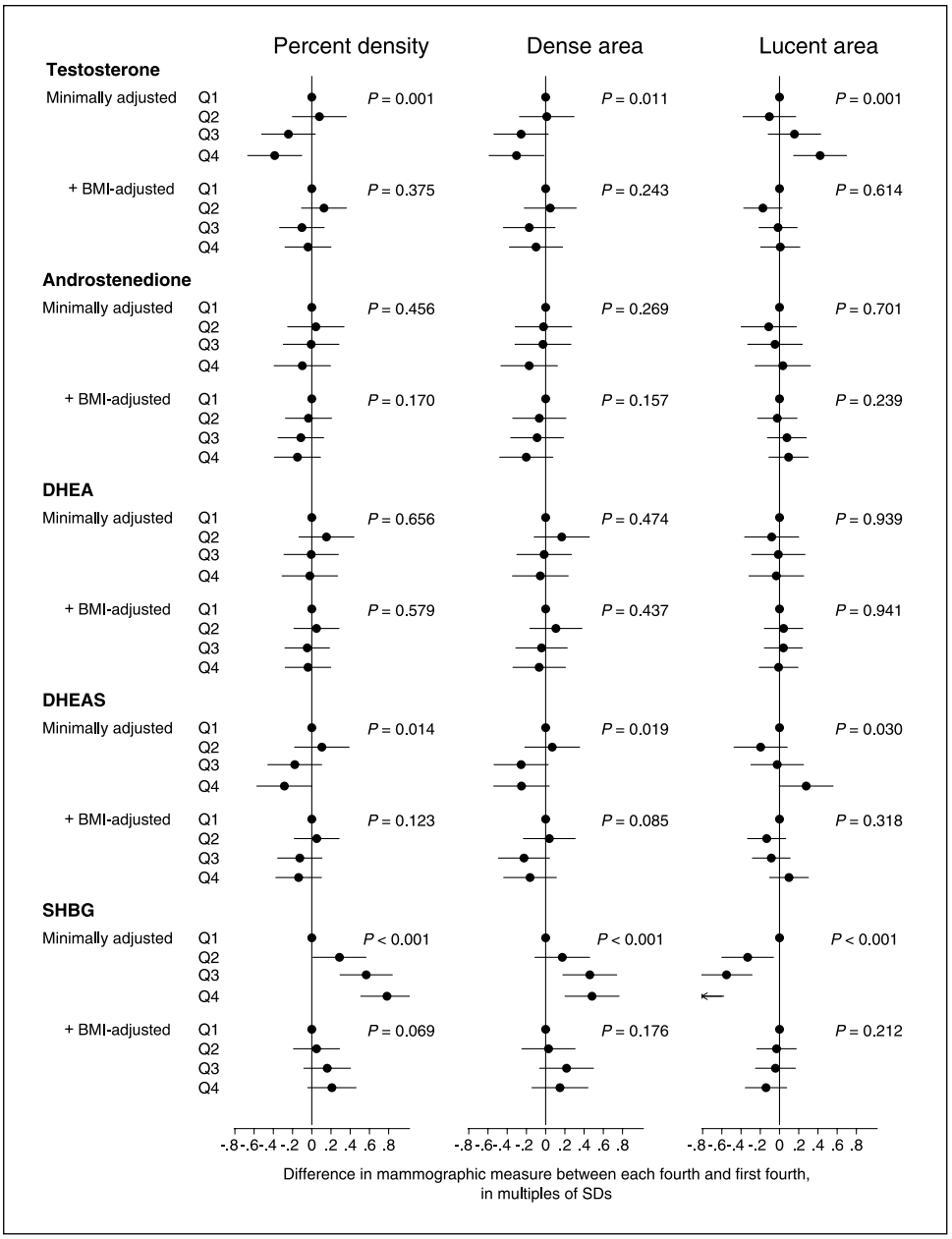


**Figure 3.** Mammographic density in relation to plasma levels of androgens and SHBG.

**Sex steroid hormones.** The most marked associations between E1G levels and percent density were observed for estimates of mean ovulatory level and daily load, with women in the top fourth of these distributions having a higher density [by 10.2% (95% CI: 2.9%, 18.1%) and 8.9% (1.7%, 16.7%), respectively] in minimally adjusted analyses (Fig. 2; Table 2; Supplementary Table S2). A similar positive association was found with AUC. Daily E1G load was estimated for each woman from her cycle length. There was weak evidence of a negative association between cycle length and density, with women with a cycle length of ≥29 days having a 4.3% (−1.7%, 9.7%) lower density than women with a cycle length of ≤27 days. The associations observed between all E1G measures and density were similar, and if anything stronger, for the subset of women with cycle lengths between 26 and 30 days.

There was only very weak evidence of an association between density and luteal E1G based on a single measurement. As luteal and mean ovulatory levels were correlated ($r$ = 0.67, on log-transformed values), the effect of luteal E1G disappeared with further adjustment for mean ovulatory levels ($P_{trend}$ = 0.67). Peak E1G was no longer significantly predictive of density after adjustment for the mean of all the other E1G measurements.

The magnitude of the various E1G-density associations was reduced on adjustment for BMI, but the positive trend in dense area persisted with both mean ovulatory levels and daily load (Fig. 2). Mean ovulatory E1G and daily E1G load were positively correlated with SHBG levels [$r$ = 0.23 ($P$ < 0.001) and $r$ = 0.22 ($P$ < 0.001), respectively]. Adjusting for SHBG did not affect the strength of the association of each E1G measure

with density in BMI-adjusted analyses. There were no clear associations between PG and any mammographic measure (Fig. 2; Table 2; Supplementary Table S2).

Minimally adjusted analyses revealed negative associations between percent density and plasma levels of testosterone, dehydroepiandrosterone sulfate (DHEAS) and, to a lesser extent, androstenedione, but they were weakened after further adjustment for BMI (Fig. 3; Table 2; Supplementary Table S3). Mutual adjustment had no further effect. None of these androgens was associated with urinary E1G levels or with plasma SHBG.

Plasma SHBG was positively associated with density (Fig. 3; Table 2; Supplementary Table S3), but adjustment for BMI attenuated this association as SHBG was correlated with this anthropometric measure ($r = 0.40$). SHBG and mean ovulatory E1G were also positively correlated ($r = 0.23$; $P < 0.001$), and adjustment for the latter further attenuated the SHBG effect on dense area ($P_{trend} = 0.19$).

**IGFs and prolactin.** Minimally adjusted analyses showed no association between plasma IGF-I levels and density. IGF-I and IGF-II levels were moderately correlated with each other ($r = 0.39$), and both were strongly correlated with IGFBP-3 levels [$r = 0.59$ and 0.70, respectively ($P < 0.001$ for all)], and the IGF-I association with density was strengthened on further adjustment for IGFBP-3 and, to a lesser extent, IGF-II (Fig. 4). Relative to women in the bottom fourth of the IGF-I distribution, those in the top fourth had an estimated 9.9% (2.9%, 17.5%) higher density (Table 2; Supplementary

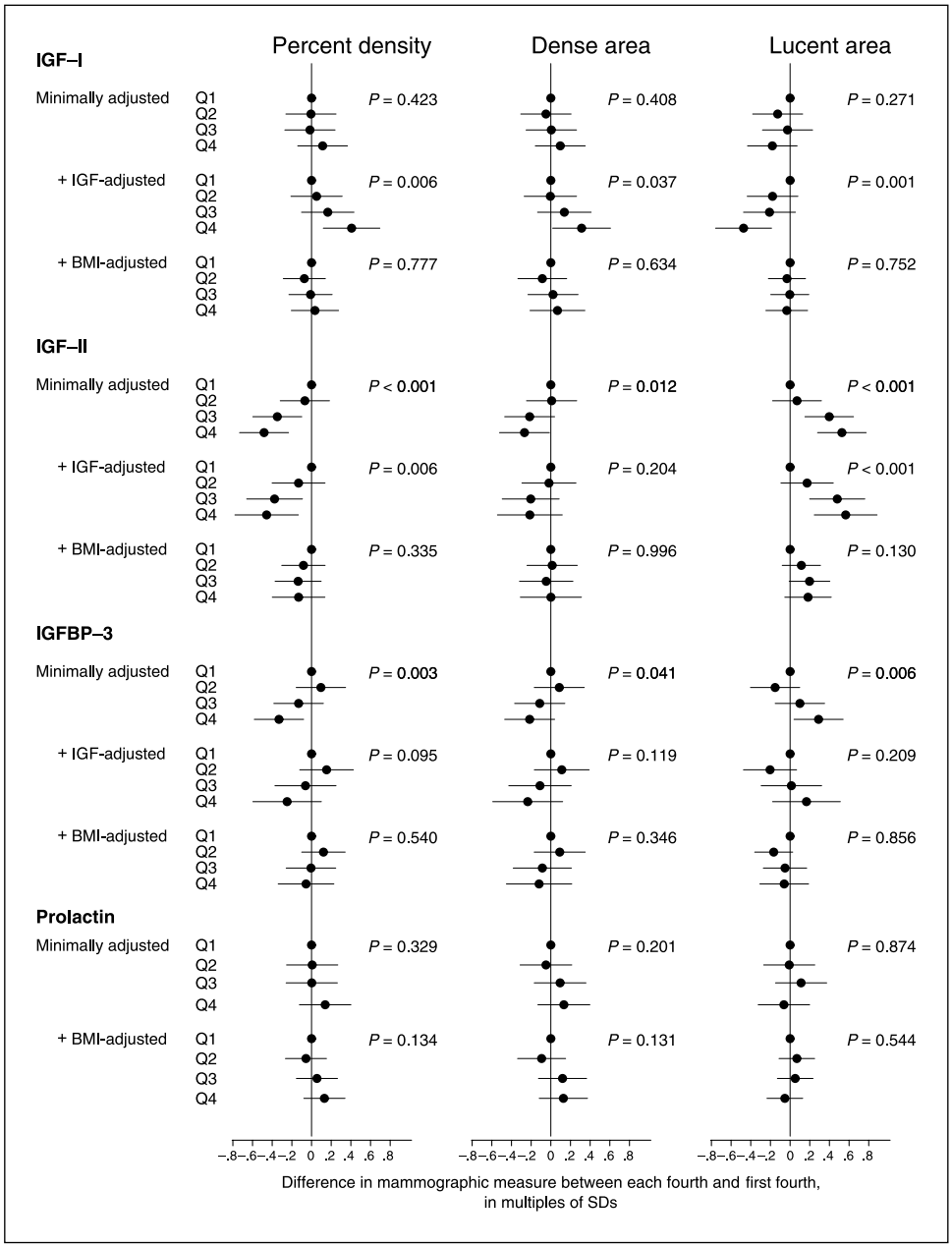


**Figure 4.** Mammographic density in relation to plasma levels of IGFs and prolactin.

Table S4). In contrast, both IGF-II and IGFBP-3 were inversely associated with percent density in minimally adjusted analyses. The effect of IGFBP-3 diminished on adjustment for the other two IGFs, but the effect of IGF-II persisted albeit mostly driven by a strong positive association with lucent area. Associations between density and each of the IGF measures disappeared on further adjustment for BMI. The associations between each of IGF-I/IGFBP-3 and IGF-II/IGFBP-3 molar ratios and density were similar to the mutually adjusted IGF-I and IGF-II effects, although not quite as strong (Supplementary Figure).

There was weak evidence of a positive association between prolactin levels and density in both minimally adjusted and BMI-adjusted analyses, with women in the highest fourth of prolactin having a 2.9% (−1.7%, 7.9%) higher density after BMI adjustment (Fig. 4; Table 2; Supplementary Table S4).

**Consistency of the findings.** Further adjustment for other potential confounders did not change the BMI-adjusted estimates for any of the hormones or growth factors (Supplementary Tables S2-S4). There was no evidence of any interactions between sex hormones and prolactin, or sex hormones and the IGFs, or between the different IGFs (results not shown).

## Discussion

This study is the first to report an association between estrogen levels and mammographic density in premenopausal women. Density was also found to be negatively associated with androgen levels but positively associated with IGF-I (after adjustment for IGFBP-3 levels) and inversely associated with IGF-II. These hormone-density associations were weakened after further adjustment for BMI with both IGF-I and IGF-II effects disappearing, but it is unclear whether BMI is acting as a confounder or as a mediator of these correlations (16).

The reliability of the mammographic measurements was very high and it was possible to examine the effects of each hormone separately on the amount of mammographic dense and lucent tissues while allowing adjustment for other correlated hormones as well as for other potential confounding factors. Although information on the time in the menstrual cycle when the mammograms were taken was not available, these variations are too small to have substantially affected our findings (17). The hormone measurements were also highly reliable. Although our participants were still ovulating, they were at the end of their reproductive life; therefore, their hormonal profiles may differ considerably from those of younger women. Hormonal levels were based on a single sample, or multiple samples from a single menstrual cycle, for each woman. These single measures may provide an imperfect estimate of a woman's long-term levels (18, 19), particularly for prolactin, which has a marked circadian variation (20). Thus, despite the high reliability of the assays and the mammographic readings, measurement errors in both might have lead to an underestimation of any true hormone-density associations. Moreover, the hormone measurements reflect circulating/urinary excretion levels and it is unclear how closely these reflect the levels of these compounds in the breast tissue, as many hormones can be produced locally and act in an autocrine/paracrine way.

**Sex steroid hormones.** Only two previous studies (3, 4) examined premenopausal estrogen levels in relation to mammographic density and their findings were inconsistent. Postmenopausal estrogens and density have been studied more extensively, but findings have been mixed (2). Our study is, however, the first to obtain serial measurements of premenopausal estrogen levels throughout the menstrual cycle, adding weight to our finding of a positive association between E1G and density. Our protocol was specifically designed to capture peak follicular phase E1G levels, but the periovulatory elevation above the average E1G level over the cycle represents only a small fraction of the total AUC, and the simplest explanation of the associations with density we observed is that the key parameter is the mean E1G level throughout the cycle. This finding would agree with Meyer and colleagues (15) who found that almost 60% of the variability in premenopausal E1G levels was explained by differences in the mean level across the cycle. A weakness of our investigation into E1G and density is that we use measurements from 7 days of a woman's cycle to estimate her total AUC. Daily E1G load incorporates information about the cycle length, and although cycle length was found to be weakly associated with E1G level, the spread in cycle length was small, and daily E1G load was not found to be any more predictive of density than mean ovulatory E1G.

Two studies reported positive associations between progesterone levels and dense area, which reduced to the null with adjustment for body size (3, 4). We observed no association between urinary PG, as measured by a single luteal sample, and density regardless of whether BMI was adjusted for.

The positive association between E1G and density is consistent with data from large prospective studies showing a positive trend in breast cancer risk with increasing levels of postmenopausal estrogen levels, but the limited research carried out into the role of premenopausal estrogen levels on breast cancer risk has been mostly null in its findings (21) perhaps because prospective studies have relied on a single prediagnostic sample from a relatively small number of cases. Our findings are, however, difficult to reconcile with trial data showing that mammographic density, in postmenopausal women, increases with use of combined estrogen-progestin hormone therapy but not with the use of estrogens alone (22). A prospective study of postmenopausal women found that both mammographic density and endogenous estrogens levels were independently associated with breast cancer risk (23); these results are not necessarily inconsistent with our findings, as density may be a better marker of lifetime cumulative exposure to endogenous estrogens than a single postmenopausal measurement of circulating levels.

Previous studies have not examined the role of androgens on breast density in premenopausal women. Our findings revealed inverse associations, which were attenuated after adjustment for BMI. These findings contrast with the positive associations between testosterone and breast cancer risk found in both premenopausal (19) and postmenopausal (24) women. Premenopausal levels of both androstenedione and DHEAS have also been found to be positively associated with breast cancer risk in some (19), but not all (25–28), studies. Together, these findings suggest that the androgen-breast cancer associations are not mediated by mammographic density (2).

In agreement with other studies (3, 4) our findings showed a positive association between SHBG and dense area, but this association disappeared after adjustment for mean ovulatory E1G, whereas the E1G effects persisted on adjustment for SHBG in BMI-adjusted analysis. It is thus plausible that SHBG may simply be acting as a marker for E1G levels.

**IGFs and prolactin.** Many studies into circulating IGF-I and density in premenopausal women found a positive association, with

or without adjustment for body size (4, 6, 7, 9), but several reasonably large studies, like ours, found no association after adjustment for BMI and other factors (3, 8, 10). Our study showed the need to adjust for IGFBP-3 when investigating the role of IGF-I, as the negative IGFBP-3 association with density masked the positive effect of IGF-I on density in unadjusted analyses. A positive association between circulating IGF-I levels and density is consistent with the finding of higher IGF-I concentrations in tissue samples from dense breasts than in samples from fatty breasts (29) and with data from a meta-analysis (30) showing a moderate positive trend in breast cancer risk with increasing premenopausal levels of circulating IGF-I, which persisted after adjustment for body size.

Previous studies on the role of premenopausal levels of IGF-II and IGFBP-3 on percent density (4–7, 9, 10) and breast cancer risk (30–34) have been inconsistent. Our study showed a negative association of IGF-II with density, independent of the effects of the other two IGFs, which diminished on adjustment for BMI, and no independent association of IGFBP-3 with density. Some of the between-study discrepancies might relate to IGFBP-3 assay methodology (35); we used a well-validated assay (36).

We found weak evidence of a positive association between prolactin and mammographic density, which was strengthened on adjustment for BMI, in contrast to two previous studies, which found no associations with percent density (4, 5), although one study reported a positive association with dense area (4). The true association is likely to be stronger as our prolactin measurements did not capture circadian variation in levels. A positive prolactin-density association would agree with a meta-analysis showing a moderate positive association between this hormone and breast cancer risk (27).

## Conclusions

The study of hormonal determinants of mammographic features is important as these will help to elucidate the biological mechanisms leading to breast cancer. It has been hypothesized that endogenous hormones could act as mitogens of breast cell proliferation, with mammographic density being a marker of this cell proliferation (2, 37). The cross-sectional design of the study makes it impossible to establish temporality, but the observed modest associations of mammographic density with E1G, prolactin, and IGF-I are consistent with the hypothesis that these hormones/growth factors may somehow promote growth of the fibroepithelial tissue in the breast. Many of these associations were attenuated with further adjustment for BMI. Further investigation is required to confirm these findings and clarify whether BMI is acting as a confounder or a mediator and to investigate whether density mediates the effect of these hormones on breast cancer risk.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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