Research Article

Cancer Epidemiology, Biomarkers & Prevention

Influence of High-Dose Estrogen Exposure during Adolescence on Mammographic Density for Age in Adulthood

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Abstract

High-dose estrogen exposure during adolescence has been hypothesized to increase a woman's breast cancer risk, possibly mediated through an increase in mammographic density, a well-established breast cancer risk factor. In 2006 to 2007, we conducted a retrospective study of women assessed for tall stature as an adolescent between 1959 and 1993. Eligible participants were ages ≥40 years and treated during adolescence with 3 mg diethylstilbestrol or 150 μg ethinyl estradiol daily or untreated. Mammograms from 167 treated and 142 untreated women were digitized. Total breast area, dense area, nondense area, and percent density were measured using a computer thresholding technique. Data on potential determinants were collected from medical records and telephone interview. Treated women had, on average, 17% lower dense area ($P = 0.032$). Means (95% confidence intervals) adjusted for age and body mass index for treated and untreated women were 24.5 cm^2 (21.8-27.2) and 29.1 cm^2 (26.0-32.4), respectively. There was no difference in adjusted means (95% confidence intervals) between treated and untreated women for nondense area [71.7 cm^2 (66.2-77.7) versus 70.5 cm^2 (64.7-76.9); $P = 0.78$], percent dense area [24.8% (22.4-27.4) versus 27.7% (24.8-30.7); $P = 0.16$], or total area [105.6 cm^2 (100.1-111.4) versus 109.3 cm^2 (103.1-115.8); $P = 0.41$], respectively. High-dose estrogen exposure during adolescence appears to curtail growth of mammographically dense tissue and therefore is unlikely to increase breast cancer risk through mechanisms related to mammographic density. *Cancer Epidemiol Biomarkers Prev; 19(1); 121–9. ©2010 AACR.*

Introduction

High-dose estrogens have been used to reduce the final height of tall girls since the 1950s (1) based on the observation that estrogen promotes the fusion of the growth plate in long bones during the latter stages of puberty (2). The estrogens most commonly used have been ethinyl estradiol and conjugated estrogens. Before 1971, diethylstilbestrol was also used but discontinued following reports that *in utero* exposure was associated with clear-cell adenocarcinoma of the vagina and cervix in the daughters of women treated during pregnancy to prevent miscarriage (3). In addition to estrogen, a progestagen was typically taken several days each month to induce cyclical bleeding.

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doi: 10.1158/1055-9965.EPI-09-0434

Treatment for tall stature has been associated with several short-term side effects on the breast such as galactorrhea, breast pain, benign breast disease, dry and cracked nipples, and deepened pigmentation of the nipples and areolae (4, 5). Suggestions that adolescent exposures might increase breast cancer risk (6, 7) have led to calls for long-term studies into the effects of high-dose estrogen treatment on breast cancer incidence (4, 8). The Australian Tall Girls Study has found that there are long-term effects of treatment on fertility (9), suggesting the possibility of long-term effects on other reproductive tissues, including the breast. The number of participants in the cohort was too small to obtain reliable estimates of any increased breast cancer risk associated with treatment.

Mammographic density is the fibroglandular tissue of the breast that appears white on a mammogram (fatty tissue appears gray) and is quantitatively measured as dense area or percent dense area. Women of the same age with higher mammographic density measures are at greater risk of beast cancer (10). Furthermore, both dense area and percent dense area for age appear to correlate over time through adulthood, with correlation estimates between measures 10 years apart in excess of 0.8 (11). Mammographic density is known to be influenced by exposure to hormones during adulthood (12, 13), although the effect might not persist long after use, raising

the possibility that hormone exposures during adolescence could have a similar effect. Because adolescence is an important stage of mammary development, exposure to high-dose estrogens at this time might also have long-term effects on breast tissue and mammographic density and hence affect breast cancer risk through this mechanism. No study has examined this hypothesis.

The aim of this study was to examine the long-term influence of high-dose estrogen treatment during adolescence on adult mammographic density measures using a retrospective cohort study of Australian tall girls to improve our understanding of the potential role of estrogen exposure during adolescence on subsequent breast cancer risk.

Materials and Methods

Participants

To be eligible for this study, women had to have previously participated in the Australian Tall Girls Study (9), be ages ≥40 years, and have stated that they were willing to be recontacted for further research. As described elsewhere (9), women who participated in the first follow-up of the Tall Girls cohort were eligible to participate if, between 1953 and 1993, they had obtained a medical opinion about their tall stature as an adolescent and had a radiologic assessment of their skeletal age to predict their adult height. This included women who had been treated with high-dose estrogens and women who had not been treated. The most common reasons for not being treated included the girl's predicted adult height did not warrant treatment, the family preferred not to have treatment, or there was little remaining growth suppression potential at the time of the assessment. The first follow-up identified a cohort of 1,432 eligible women (572 treated and 860 untreated; 1,248 from medical records including 1,222 from one pediatric endocrinologist and 184 from self-referrals), of whom 1,243 (517 treated and 726 untreated) were traced and invited to participate. Of these, 371 treated women (72% of those traced) and 409 (56%) untreated women completed a postal questionnaire and telephone interview in 2002 to 2003. The mean ages for treated and untreated women were 39.8 and 37.7 years, respectively ($P < 0.001$). Written consent to extract data from medical records was provided by 726 women (353 treated and 373 untreated) and records were available for 264 treated women (75% of those who provided their consent) and 354 untreated women (95%). More records were available for untreated women because a greater proportion of them (97%) compared with treated women (68%) were sourced from one endocrinologist who retained and allowed access to the medical records. More treated women self-referred to the study and had been treated by other endocrinologists whose records could not be accessed.

This study of mammographic density involved a second follow-up and was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee and by the relevant breast screening services across Australia. All participants gave written informed consent. This second follow-up (see Fig. 1) occurred in 2006 to 2007 and included only those from the first-follow up who were ages ≥40 years and therefore eligible to have a free mammogram as part of the national breast screening program (*BreastScreen*). The contact details of 517 eligible women (263 treated and 254 untreated) were checked and updated with information from the electronic electoral roll (registration for voting is compulsory for all adult Australians) and online telephone directory. Of these, 483 (251 treated and 232 untreated) were successfully traced and contacted. The majority of treated (94.0%) and untreated women (98.6%) were Australian-born.

Women invited into the study were asked to complete a telephone interview and to give us permission to access any mammogram they may have had in the previous 2 years or to have a mammogram at their local *BreastScreen* service and to give us permission to access the mammogram. Of those contacted, 339 (70%) provided written consent to participate (185 treated and 154 untreated) and 336 were subsequently interviewed by telephone. The mammograms of 319 women were obtained from *BreastScreen* (77% of treated and 73% of untreated), private screening services, or participants (if they held them) and returned after use. Of these, 309 were eligible for mammographic density measurement (167 treated and 142 untreated). Mammograms were ineligible if the woman had breast surgery in both breasts before the mammogram (treated = 4 and untreated = 3), if tamoxifen was used within 2 years before the mammogram (treated = 1 and untreated = 1), or if the image was of poor quality (treated = 1). Pediatric medical record data were available for those who had provided written

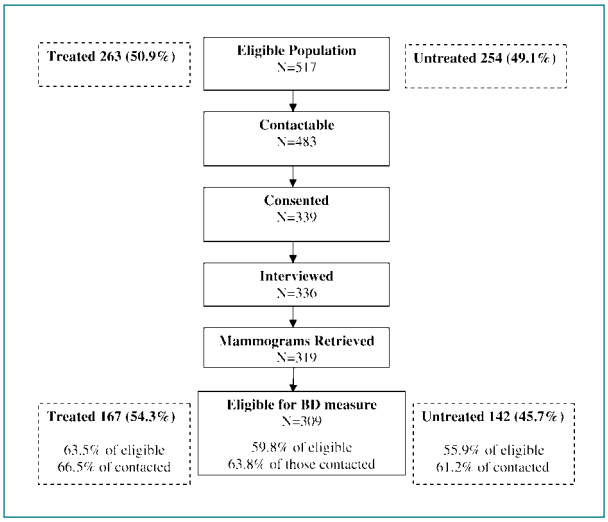

Figure 1. Eligible study population and participation by treatment status.

consent. Most (81%; 113 treated and 138 untreated) had been treated or assessed by one pediatric endocrinologist.

Exposure to Estrogen

Treatment information was extracted from the medical records of women who provided consent and for whom records were available (67% of treated and 92% of untreated women) or was self-reported by women in the postal questionnaire used in the first follow-up. Treated women received one of two types of estrogen during adolescence. Those treated before 1971 received 3 mg diethylstilbestrol daily. After that time, 150 μg ethinyl estradiol became the usually prescribed treatment. A progestagen was typically taken several days each month to induce cyclical bleeding.

Mammographic Measures

For each participant, the craniocaudal view of one mammogram was digitized by a Lumisys 85 scanner. Using a computer-assisted thresholding method described elsewhere (14), the back of the breast was outlined and the pectoral muscle and chest wall were masked to exclude it from analysis. The observer then selected a gray value as a threshold to separate the image of the breast from the background, which determined breast size. A second threshold was then selected to identify the edge(s) of the mammographically dense tissue (the area that appears white on the mammogram). The computer recorded the number of pixels within the defined areas, providing measures of total area of the breast and area of dense tissue from which nondense area and percent dense area were derived. Pixels were manually converted into cm^2 (1 pixel = 6.76×10^{-4} cm^2).

The right breast image was used (for 93.0% of treated and 92.8% of untreated women) unless surgery, cancer, or benign breast disease had occurred in that breast, in which case the left breast image was used. If the woman had a history of benign breast disease in both breasts and no surgery, the right breast image was used (n = 10 treated and n = 3 untreated). One treated woman (0.6%) and three untreated women (2.1%) had a history of breast cancer in the breast not used to measure density. One woman with a history of breast cancer had been treated with chemotherapy that can affect ovarian function. This person fulfilled the criteria for menopause (see below) and was subsequently classified as postmenopausal.

All images were measured in random order by a single trained reader (H.L.J.) and remeasured at a separate time. The intraclass correlation coefficient for repeat reads was 0.93 for both percent dense area and dense area. Means of the two sets of reads were used in the analyses.

Potential Confounders

Data on the following variables were self-reported at first follow-up (by postal questionnaire or computer-assisted telephone interview) and updated at second follow-up (by computer-assisted telephone interview): reproductive history (including pregnancy outcomes and breastfeeding history); history of breast and gynecologic disorders (e.g., endometriosis, uterine fibroids, ovarian cysts, benign breast disease, and breast cancer); use of hormonal medications, hormonal contraceptives, hormone replacement therapy (HRT), and hormones for infertility; smoking and alcohol consumption; and current height and weight [from which body mass index (BMI) was derived]. Information was obtained at first follow-up on age at menarche, highest education level achieved, and marital status. At second follow-up, participants also reported family history of breast cancer and country of birth. Women were defined as postmenopausal if the time since their last period was ≥52 weeks and (*a*) they had not started HRT before their periods ended or (*b*) they started HRT before their periods ended and they were ages ≥55 years. Recognizing that some women would have not had a period for ≥52 weeks because of hysterectomy (while retaining one or both ovaries), endometrial ablation, intrauterine device, or hormone implants, we also examined menopausal status using a definition that considered these women to be premenopausal unless they were ages ≥55 years. Menopause defined in this way resulted in 23 (13.8%) treated women and 8 (5.6%) untreated being classified as postmenopausal.

Birthweight, birth length, Tanner stage (breast; ref. 15) at time of treatment, estimated mature height (EMH) and bone age at time of pediatric assessment for treated and untreated women, and multiple height and weight measures of treated women (before, during, and, in most cases, after treatment) were abstracted from the medical records of women who had provided consent. Bone age was derived from radiographs of the wrist and the Greulich-Pyle atlas (16). EMH was calculated using the Bayley-Pinneau tables that give estimates of final adult height for normal girls according to their current height and bone age. EMH minus final height is a measure of the effectiveness of treatment at reducing final height. Bone age minus chronological age is an indicator of bone maturity at a given age. Because the age at which bone maturity is measured is of importance, bone age minus chronological age was adjusted for chronological age at time of measurement when included in a regression model.

Statistical Analyses

The outcome variables (dense area, nondense area, total breast area, and percent dense area) were treated as continuous variables and transformed to approximate normality using the Box-Cox method to choose the most appropriate transformation (17). Consequently, dense area and percent dense area were square root transformed, whereas total breast area and nondense area were log-transformed for regression analyses.

Multiple linear regression was used to assess effects of treatment. In treated women only, type of treatment (ethinyl estradiol and diethylstilbestrol), effect of duration and treatment effectiveness, and age and pubertal stage at

Table 1. Characteristics of treated and untreated participants [mean (SD) or proportions where applicable]

Characteristics	Treated (n = 167)	Untreated (n = 142)	P
Age (y), mean (SD)			
At interview	48.4 (4.8)	46.2 (4.1)	**<0.001**
At mammogram	48.0 (4.7)	45.8 (4.1)	**<0.001**
Postmenopausal* (%)	57 (34.1)	25 (17.6)	**0.001**
Age at menarche (y),† mean (SD)	12.8 (1.6)	12.8 (1.4)	0.86
Live births (%)			
0	36 (21.6)	26 (18.3)	0.48‡
1	23 (13.8)	11 (7.8)	
≥2	108 (64.7)	105 (74)	
Age at first live birth (y), mean (SD)	30.0 (4.6)	29.4 (4.5)	0.25
Height (cm), mean (SD)	178.4 (3.8)	175.6 (4.7)	**<0.001**
Weight (kg), mean (SD)	78.4 (16.2)	79.9 (15.7)	0.40
BMI (kg/m^2), mean (SD)	24.6 (5.2)	25.9 (4.9)	**0.030**
Birthweight (kg),§ mean (SD)	3.6 (0.51)	3.5 (0.49)	0.13
Birth length (cm),‖ mean (SD)	53.2 (2.5)	52.9 (2.4)	0.53
EMH-final height (cm),¶ mean (SD)	2.1 (2.8)	−1.0 (3.2)	**<0.001**
Fertility drugs (ever used; %)	43 (25.8)	18 (12.7)	**0.004**
HRT			
Ever used (%)	35 (21.0)	15 (10.6)	**0.013**
Current use (%)	22 (13.2)	7 (4.9)	**0.013**
Total use (y), mean (SD)	3.9 (6.2)	2.6 (3.1)	0.073
Hormonal contraceptive			
Ever used (%)	161 (96.4)	138 (97.2)	0.70
Current use (%)	20 (12.0)	22 (15.5)	0.37
Total use (y), mean (SD)	10.1 (7.2)	11.9 (6.7)	**0.028**
Family history of breast cancer: first-degree relative (%)	27 (16.2)	19 (13.4)	0.49
Smoking (%)			
Ever smoked	88 (52.7)	83 (58.4)	0.31
Currently smoke	18 (10.8)	17 (12.0)	0.74
Alcohol use (%)			
Never or rarely drink	24 (14.3)	24 (16.9)	0.24
Occasionally (less than once a week)	28 (16.8)	25 (17.6)	
Once or twice a week	33 (19.8)	34 (23.9)	
≥3 d/wk	82 (49.1)	59 (41.5)	
Benign breast disease (%)	37 (22.2)	23 (16.2)	0.19
Endometriosis** (%)	30 (18.0)	16 (11.3)	0.10
Total breast area (cm^2)			
Median (5th and 95th percentile range)	102.6 (49.7-232.9)	111.8 (57.4-236.0)	
Mean (SD)	114.5 (57.2)	123.9 (55.5)	0.15
Dense area (cm^2)			
Median (5th and 95th percentile range)	26.4 (3.1-60.0)	27.8 (4.7-77.6)	
Mean (SD)	27.6 (17.6)	32.8 (22.8)	**0.022**

(*Continued on the following page*)

start of treatment were examined. Least square means for each treatment were calculated from the regression coefficient estimates adjusted for mean age and BMI and number of live births. For ease of interpretability, these least square means and their confidence intervals were back-transformed.

Potential confounders (see list in Table 1) were identified from the previous literature, through being either associated with mammographic measure(s) and/or breast cancer risk, and being measured using the questionnaires and other data sources. These were entered into regression models that included treatment, starting with age

Table 1. Characteristics of treated and untreated participants [mean (SD) or proportions where applicable] (Cont'd)

Characteristics	Treated (n = 167)	Untreated (n = 142)	P
Nondense area (cm^2)			
Median (5th and 95th percentile range)	71.7 (18.5-226.9)	69.2 (27.0-225.8)	
Mean (SD)	87.0 (60.5)	91.1 (59.8)	0.55
Percent density (%)			
Median (5th and 95th percentile range)	26.8 (2.2-67.1)	28.2 (2.5-66.1)	
Mean (SD)	29.5 (20.4)	30.8 (19.6)	0.58

*Women were defined as postmenopausal if the time since their last period was ≥52 weeks and (*a*) they had not started HRT before their periods ended or (*b*) they started HRT before their periods ended and they were ages ≥55 years.
†Age at menarche: 1 missing.
‡P value for ever live birth.
§Birthweight: n = 72 treated and 112 untreated.
‖Birth length: n = 48 treated and 58 untreated.
¶EMH: n = 251.
**Endometriosis: 2 missing.

and BMI. Thereafter, order was determined by forward selection, and variables were retained if the treatment coefficient changed by ≥10%.

We examined interactions between the categorical variables age >50 years and treatment status by testing whether the coefficients for interaction were significantly different from zero when included in the model. Using Wald's test, none of the P values were <0.1.

Component and residual plots indicated nonlinearity for the regression of nondense area against BMI. Although BMI and nondense area are positively correlated, the relationship is curvilinear (the gradient is reduced at the higher end of the BMI scale). For BMI-adjusted analyses, where the response variable is nondense area, an inverse square root transformation of BMI was used to meet the assumptions of linearity. This transformation was determined using a fractional polynomial technique (18).

Birthweight, birth length, and bone age data were not complete for all women. A subgroup analysis restricted to those for whom data were available was done for these variables. Twenty-one of the mammograms were film copies of digital images and a repeat analysis was done with them removed.

Following convention, nominal statistical significance was based on $P < 0.05$. P values for differences in characteristics were calculated using t tests for means and χ^2 for proportions. All tests of significance were two-sided. Stata software (version 9) was used for all analyses.

Results

Compared with the 208 eligible women studied at first follow-up who did not participate at second follow-up, the 309 participants were almost identical in mean height, BMI, and proportion with a history of having had a breast biopsy (all $P > 0.7$). Participants were older (47.5 years) than nonparticipants (46.3 years; $P = 0.003$).

Table 1 shows that, compared with untreated women, treated women were on average older at interview and mammogram by 2.2 years ($P < 0.001$), taller by 2.8 cm ($P < 0.001$), and had a lower mean BMI by 1.3 kg/m^2 ($P = 0.030$). They were more likely to have had fertility drug treatment ($P = 0.004$), to have ever used HRT ($P = 0.013$), and to be postmenopausal ($P < 0.001$). They did not differ by age at menarche, birthweight, birth length, oral contraceptive use (ever and current use), age at first live birth, smoking and alcohol history, marital status, or educational level (data not shown).

Of the 167 treated women, 90 (53.9%) were treated with diethylstilbestrol, 62 (37.1%) with ethinyl estradiol, and 2 (1.2%) with both; for 13 (7.8%) women, the treatment type was unknown. Mean (SD) age at start of treatment was 12.8 (1.7) years and mean treatment duration was 23.6 months (21.0 months for ethinyl estradiol and 25.8 months for diethylstilbestrol). Treatment commenced after menarche for 85 (50.9%) women. Of the 103 women for whom data were available on Tanner stage of breast development, 85 (82.5%) commenced treatment at or after stage 3. The bone age of untreated women was more advanced than their chronological age; the mean difference was 0.33 years and this differed from the treated women for whom it was less than their chronological age (−0.01 years; P = <0.001).

Table 2 shows estimates of the treatment coefficients from regression models adjusting for different sets of putative determinants for each of the four mammographic measures. Table 3 shows the resulting least square means for treated and untreated women. These means show that the treated women had less dense area than untreated women overall and after adjusting for age, BMI, and any other covariates (all $P < 0.03$). After adjusting for

Table 2. Multiple linear regression of the association between treatment and each of the mammographic measures adjusted for different sets of covariates

Covariates	Regression coefficient (95% CI)	*P*
Dense area*		
None	−0.45 (−0.87 to −0.04)	0.032
Age	−0.30 (−0.72 to 0.12)	0.16
Age, BMI	−0.45 (−0.86 to −0.04)	0.032
Age, BMI, benign breast disease, and endometriosis	−0.54 (−0.95 to −0.12)	0.009
Percent density*		
None	−0.17 (−0.62 to 0.28)	0.47
Age	0.06 (−0.40 to 0.51)	0.81
Age, BMI	−0.25 (−0.64 to 0.13)	0.20
Age, BMI, live births	−0.28 (−0.67 to 0.11)	0.16
Age, BMI, live births, benign breast disease and endometriosis	−0.32 (−0.70 to 0.07)	0.11
Total breast area†		
None	−0.09 (−0.19 to 0.01)	0.070
Age	−0.12 (−0.22 to −0.01)	0.028
Age, BMI	−0.03 (−0.11 to 0.05)	0.41
Nondense area†		
None	−0.08 (−0.23 to 0.08)	0.32
Age	−0.14 (−0.30 to 0.01)	0.072
Age, BMI	0.01 (−0.11 to 0.13)	0.90
Age, BMI, live births	0.02 (−0.10 to 0.14)	0.78

NOTE: Age (years), BMI (kg/m^2), HRT (current), and menopause [postmenopausal if the time since last period was ≥52 weeks and (*a*) had not started HRT before periods ended or (*b*) started HRT before periods ended and ages ≥55 years].
*Square root transformed.
†Log transformed.

age and BMI, the difference was on average 4.6 cm^2 or 17% ($P = 0.032$). For all other mammographic measures, there was no difference between treated and untreated women before or after adjusting for potential confounders (all $P > 0.05$), although the unadjusted total breast area was marginally less for treated women ($P = 0.070$). Use of the alternative definition of menopausal status (see Materials and Methods) did not change the results for dense area, percent density, nondense area, or total breast area.

The difference in the proportion of treated and untreated women who have been diagnosed with benign breast disease (although statistically insignificant; $P = 0.19$) was not explained by age or educational level. When benign breast disease was included in the model, the association between treatment and dense area was strengthened somewhat (Table 2).

These results were not changed after removing the 21 women whose mammograms had been film copies of digital images. More treated than untreated women

Table 3. Adjusted least square mean (95% CI) of each of the mammographic measures for treated and untreated women

	Treated mean (95% CI), *n* = 167	Untreated mean (95% CI), *n* = 142
Dense area (cm^2)*	24.5 (21.8-27.2)	29.1 (26.0-32.4)
Percent density (%)†	24.8 (22.4-27.4)	27.7 (24.8-30.7)
Nondense area (cm^2)†	71.7 (66.2-77.7)	70.5 (64.7-76.9)
Total breast area (cm^2)*	105.6 (100.1-111.4)	109.3 (103.1-115.8)

*Adjusted for age (years) and BMI (kg/m^2).
†Adjusted for age, BMI, and number of live births.

self-referred themselves to the study. When we removed from the analysis women who self-referred, the results for dense area remained unchanged (coefficient, −0.49; 95% CI, −0.95 to 0.02; $P = 0.041$; $n = 253$).

After adjusting for age and BMI, the treatment regression coefficient for dense area did not differ according to type of treatment, duration of treatment, effectiveness of treatment, Tanner stage, or menarche status at the start of treatment in the treated group only (all $P > 0.30$; data not shown).

Figure 2 shows that total breast area and nondense area were negatively associated and percent dense area positively associated with age at the start of treatment (all $P < 0.001$), but there was no association with dense area ($P = 0.15$). Women who started treatment at Tanner stage 5 had a greater total breast area than those who began treatment at stage 1 or 2 when adjusted for age and BMI (regression coefficient, 0.24; 95% CI, 0.004-0.47; $P = 0.046$). After adjusting for age at start of treatment, the difference became more pronounced (regression coefficient, 0.42; 95% CI, 0.15-0.69; $P = 0.003$), suggesting that women who started treatment at a later stage in breast development had greater total breast area than those who started early.

Some girls were not treated with high-dose estrogens because their skeletal maturity (bone age minus chronological age) indicated insufficient remaining growth potential. This marker of maturity was examined as a potential confounder in a subgroup with the relevant data ($n = 237$). When adjusted for skeletal maturity (further adjusted for chronological age at time of measurement), the difference observed in square root dense area between treated and untreated women increased; specifically, the regression coefficient changed from −0.40 (95% CI, −0.88 to 0.08; $P = 0.100$) to −0.52 (95% CI, −1.01 to −0.03; $P = 0.036$). A similar effect was observed with percent dense area but not total breast area or nondense area. For percent dense area, the regression coefficient changed from −0.40 (95% CI, −0.84 to 0.05; $P = 0.081$) to −0.51 (95% CI, −0.96 to −0.05; $P = 0.028$).

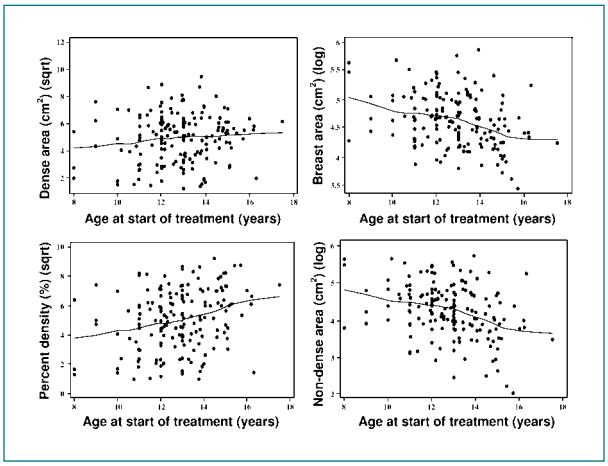

Figure 2. Lowess smoothed plots illustrating the relationship between square root dense area (cm^2), log total breast area (cm^2), log nondense area (cm^2), and percent density (%) with age at start of treatment (years).

Discussion

This study found that women treated with estrogen for tall stature during adolescence had, as adults, less dense area than women who were similarly assessed for tall stature but untreated. The magnitude of this difference did not appear to depend on the effect of treatment on epiphyseal closure and hence height reduction. Treated and untreated women did not differ in total breast area, percent dense area, or nondense area. Puberty in girls treated with high-dose estrogen is accelerated. Given that dense area for age correlates over time through adulthood, the lower mean adult dense area observed for treated women suggests less net growth in dense area during puberty. This is consistent with accelerated maturation and with the many cross-sectional observations that earlier age at menarche is associated with reduced adult mammographic density (19-23).

There are several mechanisms that might explain the findings of this study. High-dose estrogens may have a direct inhibitory effect on the developing breast in particular, the epithelial and stromal tissue that make up the mammographically dense part of the breast. This effect may be mediated by reduced levels of insulin-like growth factor-I observed in treated girls during treatment (24). Although insulin-like growth factor-I has been positively associated with mammographic density (25-27), no studies have examined the association between insulin-like growth factor-I levels during adolescence and mammographic density as an adult. Kleinberg et al. have highlighted the importance of insulin-like growth factor-I in ductal morphogenesis during pubertal mammary development in insulin-like growth factor-I insufficient animals (28).

The apparent effect of adolescent exposure to high-dose estrogens on dense area cannot be generalized to all exogenous estrogen exposures that might occur during adolescence. Estrogen has a biphasic effect on some tissues (29), exerting a different action at low concentrations than at high concentrations. For example, at low plasma concentrations, endogenous estrogen is believed to stimulate the growth spurt at the start of puberty, whereas, at higher concentrations at the end of puberty, endogenous estrogen plays a role in the cessation of growth. It may follow therefore that lower exogenous estrogen exposures during adolescence may have a different effect on mammographic density, and hence breast cancer risk, than that suggested by this study for high estrogen exposures.

No other study has examined the long-term effect of high-dose estrogen exposure during adolescence on mammographic density in adulthood. HRT is known to increase mammographic density in postmenopausal women (30), but this effect does not remain after treatment has been discontinued (31). In this study, hormone treatment in adolescence appears to have an

effect many years after the discontinuation of treatment but likely due to a different mechanism—early curtailing of growth during an important stage in breast development.

This study has some limitations. First, the findings could be prone to selection bias due to differential nonresponse in the first and second follow-ups. However, we are interested in associations not prevalence estimates, so nonresponse would be a problem only if it were systematically associated with both the predictor and the outcome variables of interest. There are no obvious and plausible reasons why women in the treated and untreated groups would vary in their willingness to participate in the study in a way that was differentially related to mammographic density. Women are generally not aware of the degree to which their breasts are dense, and dense area is not known to be highly dependent on any lifestyle factor, so they are unlikely to have been selected based on their mammographic density. Women with breast problems might have been more likely to participate, and a history of biopsies for benign breast disease has been associated with an increase in percent dense area (32, 33), but participants were no more likely to have had a breast biopsy at the time of their first follow-up than nonparticipants.

There is a growing interest in the association between childhood growth and breast cancer risk (34, 35), in particular, birthweight (36), birth length (36, 37), childhood BMI (38-42), height (38, 39, 42-44), growth velocity or age at peak height velocity (38, 40), and age at attained height (45, 46). Only a few studies have examined the association between childhood growth parameters and mammographic density (47-50). We found that treated girls differed from untreated girls for some growth parameters and attempted to address these differences by including birthweight and birth length, bone maturity, EMH, current weight, height, and BMI in the regression model, of which "only" current BMI remained. Adjusting for bone maturity at adolescence increased the treatment coefficient on dense area.

With respect to concerns about high-dose treatment during adolescence and future breast cancer risk, this study has shown that such treatment does not increase mammographic density. On the contrary, treated women in this study had an overall lower mean mammographic dense area than untreated women. Boyd et al. suggest that every 4.06 cm^2 increase in total mammographic dense area is associated with an increase of 3% in breast cancer risk (51). If true, our study would suggest that treated women were at a 3% reduced risk of breast cancer if all other risk factors remained the same.

In summary, this study provides insights into the effects on mammographic density of adolescent exposure to high-dose estrogens. It also provides some reassurance for women treated with high-dose estrogens for tall stature during adolescence—that treatment is unlikely to increase their risk of breast cancer through mechanisms related to mammographic density.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Fiona Bruinsma, Joanne Rayner, Penelope Jones, Michelle Kealy, Emma Stubbs, Shirley Catchpole, and Tim Albion for assistance with data collection and administration and Fiona Bruinsma and George Werther for feedback in the development of the grant.

Grant Support

National Health and Medical Research Council and Cancer Council Tasmania.

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Received 5/6/09; revised 10/5/09; accepted 10/21/09; published online 1/7/10.

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Cancer Epidemiol Biomarkers Prev 2010;19:121-129.