14 Exogenous and Endogenous Hormones, Mammographic Density and Breast Cancer Risk: Can Mammographic Density Be Considered an Intermediate Marker of Risk?

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Abstract Elevated mammographic density measures are a well-established, relatively strong risk factor for breast cancer development. A systematic review of prospective cohort studies and cross-sectional studies strikingly establishes parallels between the associations of combined postmenopausal estrogen and progestin replacement therapy with, on the one hand, mammographic densities and, on the other hand, breast cancer risk. Other parallel observations were the inverse associations of both mammographic density and breast cancer risk with the selective estrogen receptor modulator tamoxifen, and direct associations with prolactin. Paradoxically, however, high mammographic density has been found associated with higher risks of both estrogen- and progesterone-receptor positive (ER+/PR+) and negative (ER−/PR−) breast cancers, while hormone replacement therapy (HRT) use, but also circulating (blood) levels of androgens, estrogens, and prolactin appear to be associated more specifically to the risk of ER+ tumors. The effects of aromatase inhibitors and gonadotropin-releasing hormone agonists on breast density, as well as on breast cancer risk, still require further investigation. Regarding circulating levels of insulin-like growth factor (IGF)-I or IGFBP-3, studies did not show fully consistent relationships with mammographic density measures and breast cancer risk. In view of these various findings, it is impossible, at present, to propose mammographic density measures as an intermediate risk-related phenotype, integrating the effects of exogenous and/or endogenous hormones on the risk of developing breast cancer.

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14.1 Introduction

Human breast tissue is composed of epithelial tissue, collagen-containing stromal tissue, and adipose tissue, of which the proportions vary widely between women (Boyd et al. 1992). On

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mammographic (X-ray) images, the epithelial and stromal tissues appear as radio-dense, and adipose tissue as nondense parts (Oza and Boyd 1993). On the basis of such X-ray images, Wolfe in the 1970s proposed a classification system of mammographic tissue structures into four major parenchymal and fat tissue distribution patterns, referred to as "normal" (N1), prominent duct pattern occupying less than one-fourth (P1) or more than one-fourth (P2) of the breast volume, and "dysplastic" (DY) (Wolfe 1976). In the 1990s, more quantitative visual estimation methods were proposed for the classification of breast mammograms into six mammographic density categories (Boyd et al. 1995). Likewise, a Breast Imaging Reporting and Data System ("BI-RADS") was developed in the United States, for a visual and semiquantitative classification of breast densities into four categories of breast density. The latter system is used especially by physicians to evaluate mammograms in the context of mammographic screening for the early detection of breast tumors. In more recent years, computer-assisted, planimetric methods were developed for the quantitative determination of breast density, which nowadays is further eased by the digitization of mammographic images (Byng et al. 1998). These planimetric methods divide the total breast area on the mammogram into areas of either high or low density. Amounts of dense and nondense can then be expressed into either a relative mammographic density score, calculated as the ratio of dense tissue area divided by total breast tissue area and expressed either as a percentage (breast density%), or as the absolute area of dense mammary tissue (in cm^2).

More than 40 epidemiological studies—recently reviewed in a metaanalysis by McCormack and dos Santos Silva (2006)—have shown increases in breast cancer risk with increasing mammographic density, as assessed by Wolf's patterns, BI-RADS patterns, or planimetry. In the studies using the more quantitative, planimetric methods, relative risks of 4.50 or higher were observed for women having highly dense breasts (>75% of dense tissue) compared to women with nondense breasts (<5% dense tissue), independently of other major breast cancer risk factors, such as age, body mass index (BMI), age at first full-time pregnancy, or family history of breast cancer. In terms of the population-attributable fraction, about 40% of breast cancer occurrence can be associated with high breast densities in North American study populations (Boyd et al. 1998). In some prospective studies (Kerlikowske et al. 2007; van Gils et al. 1999), but not all (Vachon et al. 2007a), longitudinal changes in breast cancer density over periods up to 5 years have also been associated with parallel changes in breast cancer risk. Mammographic density thus appears to be one of the strongest risk factors for breast cancer, and increasingly is being proposed as an important factor in breast cancer risk prediction models (Chen et al. 2006; Vachon et al. 2007c). It is worth noting that in a number of studies (Kato et al. 1995; Maskarinec and Meng 2000) the absolute area of dense mammary tissue was found to be equally strongly associated with breast cancer risk measures as relative mammographic density measures. Measures of the absolute dense area may have the advantage of being less correlated with, or potentially confounded by, general adiposity (as discussed further in this review).

Increased density reflects increased volumes of stromal and epithelial tissues (Hawes et al. 2006), which are the mammary tissue types with the highest rates of cell proliferation. Cell proliferation rates are believed to be largely controlled by hormones (Albanes et al. 1988; Torres-Mejia et al. 2005; Trichopoulos and Lipman 1992). Furthermore, the epithelial compartment is thought to be the origin of most breast tumors, the development of which is also known to be hormone-dependent. It has thus been questioned whether mammographic density could be a useful intermediate surrogate marker for the effects of hormones on breast

cancer risk. In the present review, we summarize the results from epidemiological studies relating mammographic density measures to exogenous and endogenous sex steroid hormones, as well as circulating levels of prolactin (PRL) and insulin-like growth factor-I (IGF-I), and address the question about whether mammographic density can indeed be seen as an intermediate endpoint that would reflect influences of these hormones on breast cancer risk.

14.2 General Determinants and Correlates of Mammographic Density

Mean breast density declines with increasing age, but within a given age group shows wide between-subject variation. Age at mammography, late menarche, and late first full-term pregnancy are associated with increased mammographic densities, whereas the percentage of density decreases by about 2% with each successive pregnancy (Boyd et al. 2007). Furthermore, percent breast density decreases after menopause by 8% (Boyd et al. 2002a) with only 30% of women aged 75–79 showing mammographic densities above 50% (Stomper et al. 1996). Taken together, however, age, menopausal status, parity, and body weight jointly can account for no more than 20%–30% of the between-subject variance in mammographic density.

A larger overall proportion of between-subject variance, in fact, appears to be due to genetic factors. In studies of monozygous and heterozygous twins, the heritability was estimated to be around 60% for percent mammographic density (Boyd et al. 2002b), 65% for absolute dense area, and 66% for absolute nondense area (Stone et al. 2006). In premenopausal women, some studies (Ursin et al. 2001; White et al. 1998) but not all (Buist et al. 2006) have shown small variations in mammographic density during the menstrual cycle, with slightly increased densities during the luteal phase compared to the follicular phase (Soderqvist et al. 1997).

An early menarche, late age at first pregnancy, low parity, and late menopause are all also associated with increased risk of breast cancer. Intriguingly, however, breast cancer incidence rates do not decrease, but actually increase with advancing age, although with a higher slope before than after menopause. To resolve this apparent contradiction, the concept of breast tissue age, as opposed to chronological age, was coined (Pike et al. 1983). According to this concept, breast tissue aging starts at menarche, whereas the rate of breast tissue aging would decrease during each live pregnancy, slow further during the peri-menopausal period, and reach its lowest values after menopause (Pike et al. 1983). Adjusting for chronological age, mammographic density would reflect the degree of mammographic tissue aging that, cumulatively, a woman would have experienced, and the age-adjusted measures of mammographic tissue age would be directly related to breast cancer risk (Martin and Boyd 2008; Pike et al. 1983).

14.3 Sex Steroid Hormones and Breast Density

14.3.1 Postmenopausal Hormone Replacement Therapy

Two large-scale intervention studies—the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial and the Women's Health Initiative (WHI)—have shown increases in (percent) mammographic density among postmenopausal women who used hormonal replacement therapy (HRT) (Greendale et al. 2003; McTiernan et al. 2005). This increase was particularly clear for the use of estrogens combined with synthetic progestins (e.g., medroxyprogesterone acetate), while for estrogens alone no, or only very

moderate, increases in density were observed. These findings were fully in line with those from several large observational studies. For example, a study in Norway showed higher mammographic densities particularly among users of continuous regimens of estradiol combined with norethisterone acetate (E2/NETA) (Bremnes et al. 2007a), whereas a study in the United States showed prospective increases in density among women who started using HRT, compared to decreases in density among women that initially used HRT but then stopped its use (Rutter et al. 2001). Likewise, one study in the Netherlands showed a reduced rate of age-related reductions in percent mammographic density among women using combined (estrogen-plus-progestin) HRT use, but not among women using regimens based on estrogens alone, or users of tibolone, a 19-nortestosterone derivative with weak estrogenic, progestogenic, and androgenic activities (Van Duijnhoven et al. 2007).

Interestingly, a number of large prospective cohort studies in the United States and Europe (Bakken et al. 2004; Beral 2003; Greendale et al. 2003; Lee et al. 2005; Stahlberg et al. 2004) have also shown increases in breast cancer risk among postmenopausal women using HRT based on estrogens combined with (synthetic) progestins, but not among women using estrogens alone (Greendale et al. 2003; Lee et al. 2005), and these were confirmed in the WHI study trial (Chlebowski et al. 2003), which compared the effects of combined estrogen-plus-progestin HRT against those of HRT based on estrogens alone, and against a placebo.

14.3.2 Endogenous Sex Hormones

Prospective cohort studies have uniformly shown increased risks of breast cancer among postmenopausal women who have higher serum concentrations of androgens [dehydroepiandrosterone (DHEA), androstenedione, testosterone] and estrogens (estrone, estradiol), and lower concentrations of sex hormone-binding globulin (SHBG)—a plasmatic carrier protein that binds testosterone and estradiol with high specificity and reduces the bioavailability of these steroid hormones to their target tissues (Kaaks et al. 2005b; Key et al. 2002). In one prospective study, so far, these associations were shown to be strongest for the risk of breast tumors that express both estrogen and progesterone receptors (ER+/PR+ tumors). Furthermore, prospective studies have also shown increased breast cancer risks among premenopausal women who have higher blood levels of testosterone (Micheli et al. 2004; Kaaks et al. 2005a; Eliassen et al. 2006) and lower levels of progesterone (Micheli et al. 2004; Kaaks et al. 2005a), and one of these studies could also demonstrate an increase in risk especially of ER+/PR+ tumors in relation to more elevated serum levels of estradiol, measured during the follicular phase of the menstrual cycle (Eliassen et al. 2006).

In the light of these various relationships of endogenous sex hormones with breast cancer risk, which are particularly consistent among postmenopausal women, at least eight different research groups have also studied the cross-sectional relationships of circulating sex hormones with mammographic density measurements (Table 14.1). In statistical analyses that were unadjusted for BMI, four of these studies revealed inverse relationships of relative (percent) mammographic density with serum levels of estrone, estradiol, and free (or non-SHBG bound) estradiol (Boyd et al. 2002c; Tamimi et al. 2005; Verheus et al. 2007b; Warren et al. 2006). Furthermore, all of these studies also showed positive associations of percent mammographic density with serum levels of SHBG, and consequently, three of the studies showed negative associations with serum levels of free testosterone, unbound to SHBG. Mammographic density measures were also associated negatively with free estradiol, and positively with SHBG, in one study on premenopausal women (Boyd et al. 2002c).

Table 14.1 Summary of studies examining the correlation between endogenous sex hormones and breast density, in pre- and postmenopausal women

		% Breast density										Absolute dense breast area									
		Study size	Estrone	Estra-diol	Free estra-diol	DHEAS	Andro-stene-dione	Testo-sterone	Free testo-sterone	SHBG	Proge-sterone	Estrone	Estra-diol	Free estra-diol	DHEAS	Andro-stene-dione	Testo-sterone	Free testo-sterone	SHBG	Proge-sterone
Premenopausal																					
(Boyd et al. 2002c)	Unadjusted	193		o	–					+	+		o	–					+	o
	Adjusted for waist circumf.				o					o	o			o					o	o
Postmenopausal																					
(Aiello et al. 2005)	Unadjusted	45	Not stated																		
Never HT users	BMI adjusted		o	o	o	o	+*	o	o	o											
Past HT users	Unadjusted	43	Not stated																		
	BMI adjusted		–	–	–	–*	–	–	–	o											
(Boyd et al. 2002c)	Unadjusted	189		–	–					+	+		–*	–					+	+
Past HT users	Adjusted for waist circumf.				–					+	o			–*					+*	o
(Bremnes et al. 2007b)	Unadjusted	722	Not stated	Not stated																	
Past HT users	BMI adjusted		+*	o	o	o	o	o	o	+		+*	o	o	o	o	o	o	+		
(Greendale et al. 2005)	Unadjusted	404	o	o	o			o	–	+	o										
Past HT users	BMI adjusted		+	+	+			o	o	+*	+*										

(continued)

Table 14.1 (continued)

		% Breast density										Absolute dense breast area								
		Study size	Estrone	Estradiol	Free estradiol	DHEAS	Androstenedione	Testosterone	Free testosterone	SHBG	Progesterone	Estrone	Estradiol	Free estradiol	DHEAS	Androstenedione	Testosterone	Free testosterone	SHBG	Progesterone
(Johansson et al. 2008)	Unadjusted	226		+						+										
Never HT users	BMI adjusted			o						o										
(Tamimi et al. 2005)	Unadjusted	520	–	–	–	o	o	o	–	+	o									
Past HT users	BMI adjusted		o	o	o	o	o	o	o	o	o									
(Verheus et al. 2007b)	Unadjusted	775	–	–	–		o	–*	–	+		–*	o	o	o	o	o	o	o	
Past HT users	BMI adjusted		o	o	o	o	o	o	o	+*		No influence of BMI								
(Warren et al. 2006)	Unadjusted	1,413	–	–				–		+										
Past HT users	BMI adjusted		o	o				o		+*										

+, Positive significant correlation ($p<0.05$); –, negative significant correlation ($p<0.05$); o, no correlation; * weak correlation (p~0.1); HT, hormone therapy

The interpretation of these results is complicated by the fact that the relative mammographic density score is inherently confounded by adiposity. The denominator of this score—total breast area—is calculated as the sum of dense plus nondense breast tissue, where the area of nondense tissue predominantly reflects the amount of adipose tissue in the breast, which generally shows a strongly positive correlation (r>0.5 in many studies) with BMI or other measures of overall adiposity. Thus, measures of overall adiposity, such as BMI, but also other (e.g., metabolic and hormonal) variables that are strongly correlated with adiposity, tend to show reciprocal relationships with percent mammographic density. Among postmenopausal women, adipose tissue is the major site of synthesis of estrogens by peripheral aromatization of androgens, and BMI correlates strongly and positively with serum concentrations of both estrone and estradiol. In addition, in both pre- and postmenopausal women, increasing adiposity is associated with reduced insulin sensitivity and, due to an increase in circulating insulin levels, reduced serum levels of SHBG and increased fractions of free testosterone and estradiol unbound to SHBG. The observed direct associations of percent mammographic density with serum SHBG, and inverse associations with serum estrogens and free testosterone, could thus all be explained by the relationships of each of these variables with overall adiposity.

Statistical adjustments for BMI may remove some of the (negative) confounding of associations between serum sex hormones and SHBG with mammographic density, and indeed, in all studies that showed negative associations of percent mammographic density with serum estrogens and free testosterone and positive associations with SHBG, these associations were substantially weakened and often no longer statistically significant after statistical adjustments for BMI or waist circumference (as a measure of abdominal fat). Interestingly, in two of the studies (Bremnes et al. 2007b; Greendale et al. 2005), adjustment for BMI revealed weakly positive and statistically borderline significant associations between percent density with serum levels of estrone. Nevertheless, in these same two studies a weakly positive association of percent density with SHBG also remained, suggesting that there could have been residual negative confounding by adiposity, and that with a more complete adjustment for adiposity an even clearer positive correlation of percent density with estrone could have appeared.

Alternatively, some studies also related endogenous hormone levels to the absolute area of dense breast tissue, which does not have the inherent negative confounding by adiposity that affects the relative density measures. In these studies, no significant correlations were observed for absolute dense area with levels of either total estradiol or free estradiol (Bremnes et al. 2007b; Verheus et al. 2007b), but these two studies did suggest, respectively, either a weakly positive (Bremnes et al. 2007b) or negative (Verheus et al. 2007b) association of dense tissue area with estrone. Furthermore, a positive relationship between SHBG and absolute dense breast area was observed in two studies (Boyd et al. 2002c; Bremnes et al. 2007b), but in premenopausal women this association disappeared after adjustment for waist circumference. A third study (Verheus et al. 2007b) showed no association of SHBG with absolute dense area at all. Taken together, these studies do not suggest any clear association of total of bioavailable serum estrogens with absolute dense areas on mammographies.

Six of the studies also examined relationships of percent mammographic density with circulating levels of androgens, but globally showed no clear and consistent pattern of associations with serum concentrations of DHEA sulfate (DHEAS), androstenedione, or total testosterone, either before or after BMI adjustment (Aiello et al. 2005; Bremnes et al. 2007b; Greendale et al. 2005; Tamimi et al. 2005; Verheus et al. 2007b; Warren et al. 2006). Before adjustment for adiposity, negative correlations with percent

mammographic density were found for total testosterone (Verheus et al. 2007b; Warren et al. 2006) or free testosterone (Greendale et al. 2005; Tamimi et al. 2005; Verheus et al. 2007b), but these associations disappeared after adjustment for BMI, and also were not observed for absolute dense breast area in these same studies (Bremnes et al. 2007b; Verheus et al. 2007b).

Progesterone—the natural progestogen—was found to be positively associated with percent mammographic density only before adiposity adjustment, and unassociated with absolute dense breast area, in one study on premenopausal women (Boyd et al. 2002c). In postmenopausal women, adjusting for BMI, either a borderline positive correlation (Greendale et al. 2005) or no correlation (Tamimi et al. 2005; Boyd et al. 2002c) was observed between serum progesterone and percent mammographic density. Before adjustment for adiposity, one study showed a positive correlation (Boyd et al. 2002c), whereas two other studies showed no association (Greendale et al. 2005; Tamimi et al. 2005), between progesterone levels and percent mammographic density.

All of the above results were reported for women who were either never or former users of HRT at the time of blood donation and mammography. There were some data to suggest that between never users and former users the association of breast density with endogenous hormones could differ. Aiello et al. reported a weakly positive correlation of percent mammographic density with androstenedione, and no association with estrogens, among women who never used HRT; by contrast, this same study showed a negative correlation with androstenedione as well as with estrogens (after BMI adjustment) among past users of HRT (Aiello et al. 2005). Reasons for such possible heterogeneity between never users and past users of HRT are unclear. Users of HRT on average tend to be leaner than never users, and also the wash-out time for the effects of exogenous hormones on endogenous hormone metabolism is unclear. Further studies addressing this issue may be needed.

14.4 Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) are substances that selectively block or modulate specific parts of intracellular signal transduction of estrogen receptors (ERs) (Jordan 2007). Two well-studied SERMs are tamoxifen and raloxifene. Tamoxifen has clear antiestrogenic actions in breast cells in vitro, and became the first drug for targeted treatment of patients with estrogen-receptor positive (ER+) breast tumors. Biologically the tamoxifen-ER complex, which is very similar to the natural estrogen-ER complex, acts as a transcription factor in the cellular nucleus. Contrary to the natural complex, however, the tamoxifen-ER complex is incapable of further recruiting certain transcriptional components, which leads to lack of expression of estrogen-responsive genes, and arrest of breast cancer cell proliferation (McDonnell et al. 1995; Metzger et al. 1988). Nevertheless, some of the estrogen responses may also be preserved, depending on tissue and cell types. Thus, tamoxifen retains estrogenic effects resulting in the reduction of serum lipid profiles (lower cholesterol levels) and in the preservation of bone density in postmenopausal women. In endometrial tissue tamoxifen also retains estrogenic responses and increases the risk of endometrial cancer. Raloxifene has biological actions that are similar to those of tamoxifen, but has additional antiestrogenic effects on the uterus, and lowers incidence rates of endometrial cancer.

Several randomized prevention trials have shown reduced risks of breast cancer after longer-term treatment with tamoxifen com-

pared with placebo, with an approximate 40% reduction in breast cancer incidence overall, no significant effect for ER-negative breast cancers, and a close to 50% reduction in ER-positive cancers (Cuzick et al. 2003). Likewise, randomized trials have also shown strongly reduced risks of breast cancer among initially cancer-free, post-menopausal women treated with raloxifene to prevent osteoporosis ("MORE" trial) (Cummings et al. 1999). Further results from this trial ("CORE" study) suggested that the benefit from raloxifene may depend on endogenous estradiol levels: among postmenopausal women whose baseline serum estradiol levels were above 10 pmol/l, 4 years of raloxifene treatment resulted in a 76% reduction in breast cancer incidence compared to the placebo group, whereas women who had undetectable estradiol levels had similar breast cancer risk whether or not they were treated with raloxifene (Cummings et al. 2002). In the Study of Tamoxifen and Raloxifene ("STAR") trial, performed among women that were estimated to be at increased risk of breast cancer, tamoxifen and raloxifene treatments resulted in equivalent reductions in breast cancer incidence rates, but raloxifene was associated with lower incidence rates of endometrial cancer and hyperplasia, cataracts, and thromboembolic events (Vogel et al. 2006).

Few studies have been published on the relationship between SERMs and mammographic density (Table 14.2). Tamoxifen caused a decrease of percent density, when administered to breast cancer patients (Atkinson et al. 1999) or to women who are at increased risk of developing breast cancer (Brisson et al. 2000; Chow et al. 2000; Son and Oh 1999). In the largest study ("IBIS-I" trial), among 388 women having a minimum initial breast density of 10% and an estimated twofold increased risk to develop breast cancer, a 5-year treatment with tamoxifen reduced breast density by 8% (Cuzick et al. 2004). Two-thirds of this overall breast density reduction was observed during the first 18 months of treatment, which made the investigators of this study speculate that breast density could be used as an early marker for prevention efficacy during tamoxifen treatment (Cuzick et al. 2004). Studies on raloxifene, however, have not shown so far any clear effects on mammographic density (Christodoulakos et al. 2002; Freedman et al. 2001; Jackson et al. 2003; Lasco et al. 2006; Table 14.2).

Taken together, these studies suggest that selective ER modulators, particularly tamoxifen, may reduce mammographic density. Unfortunately, it was not examined directly in these studies whether such reductions are indeed the result of diminished absolute dense tissue areas, although a priori this would seem likely. Although raloxifene showed less clear effects on mammographic density, data available from these first studies are still insufficient to draw definitive conclusions of an absence of effect on breast density. One major question, in this context, is whether the effects on breast density by raloxifene, but also tamoxifen, are modulated by blood concentrations of endogenous estrogens.

14.5 Aromatase Inhibitors

Aromatase inhibitors are effective alternatives to selective ER modulators for the treatment of estrogen-receptor positive breast cancer, and are also being studied as possible chemopreventative agents against breast cancer, among women at high risk for this disease. Letrozole and anastrozole belong to the group of reversible nonsteroidal imidazoles, and exemestane to the class of irreversible steroidal inhibitors. Aromatase inhibitors are being utilized as initial hormonal therapy on localized hormone receptor-positive breast cancer patients

Table 14.2 Summary of studies examining the correlation between tamoxifen, raloxifene, and relative breast density measurements

Author	Study population	Study size	Duration of treatment	Association
	Tamoxifen			
(Atkinson et al. 1999)	Cancer patients	94	Not stated	–14%
(Atkinson et al. 1999)	Cancer free	188	Not stated	o
(Brisson et al. 2000)	Cancer free, high risk	36	5 years	–9%
(Chow et al. 2000)	Cancer free, high risk	28	2 years	–4.3%/year
(Cuzick et al. 2004)	Cancer free, high risk	388	5 years	–8%
(Son and Oh 1999)	Cancer patients	102	22 months	– (More prevalent in pre than post)
			Raloxifene	
(Christodoulakos et al. 2002)	Cancer free, high risk for CVD or osteoporosis	48	12 months	o
(Freedman et al. 2001)	Cancer free, hysterectomy	87	2 years	–2%
(Jackson et al. 2003)	Postmenopausal women with osteopenia or osteoporosis	109	12 months	o
(Lasco et al. 2006)	Healthy	70	2 years	–

o, no association, –, negative association; CVD, cardiovascular disease

(Howell et al. 2005), as a switching reagent in the 5-year follow-up treatment after 2 to 3 years of taking tamoxifen (Coombes et al. 2004) and as extended adjuvant hormone therapy after 5 years treatment with tamoxifen (Goss et al. 2003). Compared to tamoxifen or a placebo, aromatase inhibitors were found to improve disease-free survival of breast cancer patients, with a 70%–80% reduction of new ER-positive breast cancer recurrences (Cuzick 2005).

So far, only very few studies have examined the effect of aromatase inhibitors on mammographic density. No significant mammographic changes were observed after 6 months of letrozole treatment in postmenopausal women at high risk of breast cancer who were on HRT, although this treatment did cause a 66% reduction in epithelial cell proliferation rates as measured by Ki-67 concentrations (Fabian et al. 2007). Comparable results were found for women with early-onset breast cancer that had first been treated with tamoxifen for 5 years (Vachon et al. 2007b).

14.6 Gonadotropin-Releasing Hormone Agonists

Based on the hypothesis that cyclic ovarian production of estradiol and progesterone accounts for the steep rise of breast cancer risk within increasing age among premenopausal

women, gonadotropin releasing hormone agonists (GnRHA) have been proposed as potentially chemopreventative agents against breast cancer (Pike et al. 1989). This type of agonist can drastically reduce ovarian sex steroid synthesis by blocking the pituitary release of luteinizing hormone. To prevent deleterious effects of estrogen deficiency, the addition of low-dose HRT to the GnRHA appears necessary. In a small randomized trial among 21 premenopausal women predisposed to familial breast cancer, aged 25–40 years, significant reductions in percent mammographic density were seen as a response to the reduced estrogen and progestogen exposures achieved after 12 months of treatment with a combined hormonal regimen consisting of GnRHA (leuprolide acetate depot by monthly intramuscular injections) combined with oral add-back administration of estrogens and progestins (Spicer et al. 1994). An extended follow-up of this study showed that the change in percent density persisted through 12 months of treatment (Gram et al. 2001). A similar, very small study, conducted by the same research group among eight premenopausal women carrying a *BRCA1* mutation, also showed a reduction in mammographic density in response to the GnRHA deslorelin, jointly administered with low-dose add-back steroids (estradiol, testosterone, intermittent medroxyprogesterone acetate) (Weitzel et al. 2007).

No studies have been conducted, so far, to examine whether GnRHA can reduce breast cancer occurrence among healthy women. Among premenopausal breast cancer patients, however, the addition of goserelin to standard adjuvant therapy was shown to be more effective than standard therapy alone, reducing breast tumor recurrence and improving survival (Baum et al. 2006).

These data show that the addition of goserelin to standard adjuvant therapy is more effective than standard therapy alone in premenopausal women with early breast cancer.

14.7 IGF-I and Its Binding Proteins

Serum levels of IGF-I have been associated with breast cancer risk in a number of prospective cohort studies, although observations are not entirely consistent (Allen et al. 2005; Gronbaek et al. 2004; Kaaks et al. 2002; Krajcik et al. 2002; Muti et al. 2002; Schernhammer et al. 2006; Toniolo et al. 2000). While initially such associations were reported particularly for breast cancer occurrence in premenopausal women (Allen et al. 2005; Krajcik et al. 2002; Muti et al. 2002; Toniolo et al. 2000), these reports were not uniformly confirmed by subsequent studies (Gronbaek et al. 2004; Kaaks et al. 2002; Schernhammer et al. 2006), and some studies also showed increased risks only among older women (Rinaldi et al. 2006). Nevertheless, the hypothesis that higher circulating levels of IGF-I could increase breast cancer risk remains plausible, as experiments in vitro have clearly demonstrated growth-promoting and antiapoptotic effects on mammary tumor cells (Ng et al. 1997). It has been hypothesized that elevated IGFBP-3 levels might reduce breast cancer risk, either by reducing the biological availability of IGF-I to cellular IGF-I receptors, or by independent pro-apoptotic effects through putative IGFBP-3 specific binding sites on cellular membranes (Pollak 2000; Yu and Rohan 2000). In a number of epidemiological studies IGF-I levels were associated with risk of cancer only when risk models included statistical adjustment terms for levels of IGFBP-3, or when the IGF-I/IGFBP-3 molar ratio was considered. Again, however, this observation has not been made uniformly across all studies, and there is some evidence that the relationship of IGFBP-3 with breast cancer risk, as well as the effects of statistical adjustments for IGFBP-3 on estimated relationships of risk with IGF-I, could be dependent on the type of immunoassay used for IGFBP-3 (Rinaldi et al. 2005). IGFBP-3 is

a complex molecule that occurs in the circulation in a variety of forms.

Among premenopausal women, cross-sectional studies showed either a positive association (Boyd et al. 2002c; Burshell et al. 2008; Diorio et al. 2005) or no association between IGF-I and relative mammographic density (Maskarinec et al. 2003; Verheus et al. 2007a) (Table 14.3), and in one of these studies IGF-I correlated positively also with absolute dense areas. IGFBP-3 levels, by contrast, were found to be negatively associated with mammographic density (Byrne et al. 2000a; Diorio et al. 2005; Maskarinec et al. 2003) after adjustment for adiposity variables, with the exception of one study (Boyd et al. 2002c). In three studies, the IGF-I/IGFBP-3 ratio showed a positive correlation with both relative breast density (Byrne et al. 2000a; Diorio et al. 2005; Maskarinec et al. 2003), and in one study this ratio showed only a weak and only borderline significant ($p < 0.1$) correlation with absolute dense breast area (Maskarinec et al. 2003; Verheus et al. 2007a).

Among postmenopausal women, mostly no correlations were found between IGF-I and percent mammographic density (Aiello et al. 2005; Byrne et al. 2000a; Diorio et al. 2005; Johansson et al. 2008). Five different studies also showed no relationship of IGFBP-3 with either percent density (Aiello et al. 2005; Boyd et al. 2002c; Bremnes et al. 2007c; Byrne et al. 2000b; Diorio et al. 2005) or absolute breast density (Boyd et al. 2002c; Bremnes et al. 2007c) with the exception of one study (Johansson et al. 2008). Divergent findings, showing positive [past HT users (Bremnes et al. 2007c)], negative [past HT users (Aiello et al. 2005)], and no correlation [never HT users (Aiello et al. 2005; Byrne et al. 2000a; Diorio et al. 2005; Johansson et al. 2008)], were published for the ratio of IGF-1/IGFBP-3 and percent breast density after BMI adjustment.

As in the case of SHBG and endogenous estrogens, adiposity is a potential confounder of the relationships of IGF-I, IGFBP-3, or their molar ratio, with percent breast density (Kaaks 2005). An elevated BMI is generally associated with a modest decrease in plasma IGF-I concentrations, with moderately increased levels of IGFBP-3, and thus with a reduced IGF-I/IGFBP-3 ratio. Since BMI also correlates inversely with relative mammographic density measures, one would expect a weak positive correlation of relative mammographic density measures with IGF-I, and especially the IGF-I / IGFBP-3 ratio. In the study by Maskarinec et al. (2003), a weakly positive correlation ($r = 0.13$) between the IGF-I/IGFBP-3 molar ratio and the percentage of mammographic density could be entirely accounted for by the direct correlation of IGFBP-3 ($r = 0.20$), and hence, the inverse correlation of the IGF-I/IGFBP-3 ratio ($r = -0.19$), with the nondense area.

14.8 Prolactin

PRL, a pituitary hormone, is important for mammary epithelial cell proliferation and differentiation and initiates lactation at higher concentration levels. It is further involved in the glandular breast development during pregnancy. Studies at the cellular level in vitro, and in vivo with multiple transgenic and knockout models have confirmed a role for PRL in breast cancer development (Clevenger et al. 2003; Harris et al. 2004). In parallel, prospective epidemiological studies have shown a positive association between PRL and breast cancer risk in both pre- and postmenopausal women, with a 30%–40% increases in risk comparing highest vs lowest quartile levels (Tworoger and Hankinson 2008). Other, smaller studies (Wang et al. 1992; Kabuto et al. 2000; Helzlsouer et al. 1994; Manjer et al. 2003) showed similar results, although findings were not always statistically significant. The association of PRL levels with

Table 14.3 Summary of studies examining the correlation between IGF-I, its binding protein 3, and prolactin with breast density measurements in pre- and postmenopausal women

			% Density				Absolute dense breast area				Absolute nondense breast area			
		Study size	Prolactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3
Premenopausal														
(Boyd et al. 2002c)	Unadjusted	193	o	+	–		o	+	–					
	Adjusted for waist circumf.		o	+	o		o		o					
(Byrne et al. 2000a)	Unadjusted	65		+*	o									
	BMI adjusted			+	–	+								
(Diorio et al. 2005)	Unadjusted	783		–	+									
	BMI adjusted			+	–	+*								
(Maskarinec et al. 2003)	Unadjusted	263		+*	–	+		o	o	+		o	+	–
	BMI adjusted			o	–*	+		o	o	+*		o	o	o
(Verheus et al. 2007a)	Unadjusted	684	Not stated			Not stated			Not stated					
	BMI adjusted			o				o				o		
Postmenopausal														
(Aiello et al. 2005)	Unadjusted	45	Not stated											
Never HT users	BMI adjusted			o	o	o								
Past HT users	Unadjusted	43	Not stated											
	BMI adjusted				o	–								
(Boyd et al. 2002c)	Unadjusted	189	+	+	o		+	+	o					
Past HT users	Adjusted for waist circumf.		+		o		+		o					
(Bremnes et al. 2007c)	Unadjusted	553	Not stated			Not stated								
Never HT users	BMI adjusted			+	o	+*		+	o	+*				
past HT users	Unadjusted	170	Not stated			Not stated								
	BMI adjusted			+	o	+		+	o	+				

(continued)

Table 14.3 (continued)

			% Density				Absolute dense breast area				Absolute nondense breast area			
		Study size	Prolactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3	Pro-lactin	IGF-I	IGFBP–3	IGF-I/ IGFBP–3
(Bremnes et al. 2007b)	Unadjusted	722	Not stated			Not stated								
Past HT users	BMI adjusted		o				o							
(Byrne et al. 2000a)	Unadjusted	192		o	o	o								
Never HT users	BMI adjusted			o	o	o								
(Diorio et al. 2005)	Unadjusted	783		+	o	+								
Never HT users	BMI adjusted			o	o	o								
(Greendale et al. 2007)	Unadjusted	400	+											
Past HT users	BMI adjusted		+											
(Johansson et al. 2008)	Unadjusted	226	o	o	–	+*								
Never HT users	BMI adjusted		o	o	–*	o								
(Tamimi et al. 2005)	Unadjusted	520	+*											
Past HT users	BMI adjusted		+											

+, positive significant correlation ($p<0.05$); –, negative significant correlation ($p<0.05$); o, no correlation; * weak correlation ($p\sim0.1$); HT, hormone therapy

breast cancer risk appears to be specific for steroid hormone-sensitive tumors (Tworoger and Hankinson 2008).

Regarding mammographic densities, one study showed no correlation of serum PRL levels with either relative or absolute breast density measures among premenopausal women (Boyd et al. 2002c). However, in postmenopausal women not currently using exogenous HRT, positive relationships for relative breast density as well as absolute dense breast area before and after BMI adjustment were published in a number of studies (Boyd et al. 2002c; Greendale et al. 2007; Tamimi et al. 2005), although two further studies showed no such correlations (Bremnes et al. 2007b; Johansson et al. 2008).

Table 14.4 Hormonal exposures, mammographic density and breast cancer risk

	Breast cancer	Breast density
HRT (E+P)	↑	↑
Tamoxifen	↓	↓
Plasma prolactin	↑	↑
Plasma sex steroids	↑	o
Aromatase inhibitors	↓	–
GnRHA	–	Possibly ↓
IGF-I	↑	o

14.9 Discussion

We have reviewed observed relationships between hormonal exposures, measures of mammographic density, and breast cancer risk, with the aim to examine whether mammographic density measurements could be seen as a potential intermediate marker of hormonal influences on breast cancer risk. Key observations include some striking parallels between the associations of combined postmenopausal estrogen and progestin replacement therapy with, on the one hand, mammographic densities and, on the other hand, breast cancer risk. Further, equally interesting parallels are the inverse associations of both mammographic density and breast cancer risk with the selective ER modulator of tamoxifen, and direct associations with PRL (Table 14.4). The effects of aromatase inhibitors and gonadotropin-releasing hormone agonists on breast density, as well as on breast cancer risk, still require further investigation.

At first sight, the interesting parallel findings for HRT use may suggest that the combination of estrogens and progestins enhance breast tumor development through pathways—e.g., enhanced cell proliferation—that simultaneously are reflected by increased mammographic densities. However, several observations would seem to challenge this view. In the Nurses' Health Study cohort (Tamimi et al. 2007; Ziv et al. 2004), but also in the study of the San Francisco Mammography Registry (Tamimi et al. 2007; Ziv et al. 2004), increased mammographic density was associated with higher risks of both ER+/PR+ and ER−/PR− breast cancers. These findings are in stark contrast with observations that combined HRT use (Chen et al. 2006; Fournier et al. 2008; Kumar et al. 2007; Li et al. 2003), but also circulating (blood) levels of estrogens (Eliassen et al. 2006; Missmer et al. 2004; Tamimi et al. 2007) and PRL (Tworoger et al. 2004; Tworoger et al. 2007) among both pre- and postmenopausal women, are related specifically to the risk of ER+ tumors.

The findings from tamoxifen intervention studies, showing reductions in both breast cancer occurrence and mammographic density in the tamoxifen intervention groups, strongly suggest a role for estrogens in the regulation of breast epithelial and/or stromal proliferation patterns, as well as in breast tumor promotion. Again, however, this reduction in tumor occurrence appears to be specific to ER+ tumors (Fisher et al. 2005). A further contrasting finding is that in cross-sectional studies there is no

clear evidence for a positive association of circulating estrogens with mammographic density, although observations from a few studies suggested that this lack of association could have been due to (residual) confounding by adiposity. Besides the estrogens, there is a total absence of association between circulating total or bioavailable androgens and mammographic density measures, again in stark contrast with observations from prospective cohort studies that found elevated serum androgens (androstenedione, testosterone) are associated with increased risks of breast cancer among both pre- and postmenopausal women.

Taken together, these various observations might lead to speculation about whether the effects of estrogen-plus-progestin HRT regimens on mammographic densities could be unrelated to the mechanisms by which such regimens increase breast cancer risk. Both the observational and intervention studies have indicated relatively acute changes in mammographic density upon either starting or stopping HRT use. It has been speculated whether these effects might be due specifically to the progestogenic component, which might cause the intralobular tissue to loosen and to become more edematous (Campagnoli et al. 2005), as also occurs naturally during the menstrual cycle. According to this speculation, mammographic density variations might merely reflect differences in tissue water content, but would be necessarily related to the types of physiological changes (e.g., in tissue proliferation and/or apoptosis) that might enhance tumor development.

An alternative speculation would be that combined estrogen-plus-progestin HRT regimens do enhance breast tumor development, but largely through mechanisms that are independent of estrogen and/or progesterone receptors. One well-documented effect of combined (oral) HRT regimens including synthetic progestins is their capacity to reduce the hepatic synthesis and circulating levels of IGF-I (Campagnoli et al. 2005). From a physiological perspective, however, such decrease in IGF-I would be expected to reduce the risk of breast cancer, and possibly also mammographic density, which is not what the majority of epidemiological studies have shown.

Regarding serum IGF-I levels, findings are not fully consistent with respect to breast cancer risk, with some studies showing an increase in risk only among premenopausal women, others only among older women, and some studies showing no relationship at all. Likewise, cross-sectional relationships did not uniformly show a direct association between circulating IGF-I and mammographic densities, although results from some studies did suggest a possible relationship especially among premenopausal women.

It is perhaps for PRL that findings are most coherent, so far, with clear positive associations of serum PRL levels with both mammographic density and breast cancer risk, among both pre- and postmenopausal women. The number of studies showing these associations, however, is still relatively small.

In summary, although there are some intriguing parallel findings relating exogenous hormones (estrogen-plus-progestin HRT), tamoxifen, or endogenous hormones (PRL) to both increased mammographic density and increased breast cancer risk, there are also major discrepant observations regarding the role of sex hormones in the regulation of mammographic density. It is therefore impossible, at present, to propose mammographic density measures as an intermediate measure of risk, integrating the effects of exogenous and/or endogenous hormones on breast tumor development.

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