Association of serum sex steroid receptor bioactivity and sex steroid hormones with breast cancer risk in postmenopausal women

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Abstract

Postmenopausal women with elevated serum sex steroids have an increased risk of breast cancer. Most of this risk is believed to be exerted through binding of the sex steroids to their receptors. For the first time, we investigate the association of estrogen receptor (ER) and androgen receptor (AR) serum bioactivity (SB) in addition to hormone levels in samples from women with breast cancer collected before diagnosis. Two hundred postmenopausal women participating in the UK Collaborative Trial of Ovarian Cancer Screening who developed ER-positive breast cancer 0.6–5 years after sample donation were identified and matched to 400 controls. ER and AR bioassays were used to measure ERα, ERβ, and AR SB. Androgen and estrogen levels were measured with immunoassays. Subjects were classified according to quintiles of the respective marker among controls and the associations between SB and hormones with breast cancer risk were determined by logistic regression analysis. ERα and ERβ SB were significantly higher before diagnosis compared with controls, while estrogens showed no difference. Women had a twofold increased breast cancer risk if ERα SB (odds ratio (OR), 2.114; 95% confidence interval (CI), 1.050–4.425; \( P = 0.040 \)) was in the top quintile \( > 2 \) years before diagnosis or estrone (OR, 2.205; 95% CI, 1.104–4.586; \( P = 0.029 \)) was in the top quintile \( > 2 \) years before diagnosis. AR showed no significant association with breast cancer while androstenedione (OR, 3.187; 95% CI, 1.738–6.044; \( P = 0.0003 \)) and testosterone (OR, 2.145; 95% CI, 1.256–3.712; \( P = 0.006 \)) were significantly higher compared with controls and showed a strong association with an almost threefold increased breast cancer risk independent of time to diagnosis. This study provides further evidence on the association of androgens and estrogens with breast cancer. In addition, it reports that high ER but not AR SB is associated with increased breast risk \( > 2 \) years before diagnosis.

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Introduction

Breast cancer remains one of the leading causes of cancer death among women despite the huge progress that has been made in treatment (Santen et al. 2007, Weigel & Dowsett 2010). Many risk factors for postmenopausal breast cancer are suggested to mediate their effect through a hormonal mechanism (Henderson & Feigelson 2000). The largest meta-analysis combining nine prospective studies demonstrated that postmenopausal women with serum estrogen and...
androgen levels in the highest quintiles have a twofold increased risk of breast cancer (Key et al. 2002). Since then, a number of studies have reported conflicting results on the association of serum sex steroid hormones and breast cancer risk (Lamar et al. 2003, Manjer et al. 2003, Onland-Moret et al. 2003, Missmer et al. 2004, Zeleniuch-Jacquotte et al. 2004, 2005, Kaaks et al. 2005, Tworoger et al. 2005, Adly et al. 2006, Beattie et al. 2006, Eliassen et al. 2006, Sieri et al. 2009, Baglietto et al. 2010). All these reports have used conventional immunoassays to measure hormone levels. In the past few years, bioactivity assays for steroid hormone receptors have been described, enabling quantification of total hormone action (Paris et al. 2002, Sievernich et al. 2004, Roy et al. 2006). As estrogen and androgen hormones exert their effects through binding to sex steroid hormone receptors, we previously hypothesized that bioactivity assays might be an attractive alternative for breast cancer risk assessment. We found that estrogen receptor α (ERα) and ERβ serum bioactivity (SB) are independently associated with breast cancer using samples collected at diagnosis (Widschwendter et al. 2009).

To better understand the long-term effect of sex steroids and bioactivity of their receptors on breast cancer risk, it is crucial to examine levels many years before diagnosis. We were able to explore this issue using the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) biobank. Women recruited to the trial between 2001 and 2005 provided blood samples for secondary studies and continue to be followed up by cancer registration and self-reporting (Menon et al. 2008, 2009). We report on a nested case-control study using serum samples donated between 6 months and 5 years before diagnosis by women who developed breast cancer after joining the trial and healthy women who had not developed the disease. SB of ERα and ERβ and androgen receptor (AR) were measured using a yeast-based assay along with five sex steroid hormones (estradiol (E2), estrone, androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS)), free E2 (fE2) and free testosterone (fT; calculated by the mass action law), and sex hormone-binding globulin (SHBG) using conventional immunoassays to examine their association with breast cancer risk.

Materials and methods

Cohort

The subjects were participants in the UKCTOCS, a multicenter randomized controlled trial of ovarian cancer screening in England, Wales, and Northern Ireland, coordinated by the Gynecological Cancer Research Centre at University College London (UCL). Women aged 50–74 were recruited through random invitation from age/sex registers of 27 participating Primary Care Trusts. At recruitment, each woman donated a blood sample, filled in a baseline questionnaire, and provided written consent giving permission to access their medical records and use their data/samples in future studies. The questionnaire included questions on demographics, height, weight, parity, hysterectomy, tubal ligation, treatment for infertility, contraceptive pill, hormone replacement treatment (HRT), and previous history of any cancer and family history of ovarian/breast cancer (Menon et al. 2008).

Selection of the study sample

All participants are being followed up through a ‘flagging study’ with the NHS Information Centre for Health and Social Care. Up-to-date cancer registration data were obtained from the agencies on 2nd February 2009 (median follow-up 5.681 years and interquartile range (IQR), 1.284 years). For confirmation of diagnosis, their treating physician was sent a questionnaire requesting information regarding their diagnosis (histology) and treatment. Two hundred women who developed ER-positive invasive breast cancer after joining the UKCTOCS and were not on HRT treatment at recruitment and had donated a serum sample between 6 months and 5 years before diagnosis were chosen as ‘cases’ for this study. Each breast cancer case was age matched with two women who had no history of breast cancer (controls) at last follow-up and had donated serum samples on the same day and in the same clinic. The UKCTOCS was approved by the UK North West Multicentre Research Ethics Committees (North West MREC 00/8/34). Ethical approval for this nested case-control study was obtained from the Joint UCL/UCLH Committees on the Ethics of Human Research (22nd February 2007, 06/Q0505/102).

Serum sample processing

The blood samples were collected into Griener Bio one gel tubes (Cat no: 455071) at the centers, shipped overnight to the central laboratory, and centrifuged at 2000 g for 10 min. The serum was removed from the cells within 56 h of sample collection and was frozen using a two-stage freezing process: 12 h at −80 °C and then placed in liquid nitrogen (vapor phase) at −180 °C. A novel semi-automated system aliquoted serum in 500 µl straws was then heat sealed, bar coded,
Sex steroid hormone receptor bioactivity using bioassay systems

Sex steroid hormone receptor bioactivity was measured using a yeast-based reporter gene assay that not only determines whether a chemical binds to the receptor, but also whether estrogen- or androgen-dependent gene expression is stimulated. The assay has been described previously (Widschwendter et al. 2009). Briefly, the genetically modified yeast cells were incubated in a defined test medium with the reference substance E2 for ERα and ERβ and dihydrotestosterone for AR test samples and negative controls. At the end of the incubation period the developed green fluorescence was determined and corrected for cell density, optical density (OD) of the cell suspension and blanks. The cell growth was determined by measuring the light absorption at 600 nm and GFP-fluorescence by measuring GFP at 535 nm, specific OD and fluorescence at $t=0$ and $t=16.5$ h for ERα and ERβ and $t=24$ h for AR in each of the 96 wells. Tests were considered as valid if the turbidity of the negative control culture increased five times during the incubation period. The control culture showed no fluorescence. The bioactivity was determined by comparison of the fluorescence development in test cultures vs the calibration curve. The dose–response curves of the reference values were fitted using the Hill equation fit and the R-function. The analysis was performed blind and cases and controls were randomly mixed. Tests were carried out with two replicates at a time on two different days (four readings in total). The lower detection limit for the ER SB is 5 pg/ml and for AR SB is 0.2 ng/ml. The inter-assay coefficients of variation were lower than 20%.

Hormone levels using immunoassay systems

For E2, testosterone, DHEAS, and SHBG kits were obtained from Roche and the samples were run on an Elecsys 2010 analyzer (Roche Diagnostics GmbH). Androstenedione levels were measured using an ELISA kit on DPC IMMULITE 2500 analyzer (Siemens Medical Solutions Diagnostics, Munich, Germany). For estrone ELISA kit was obtained from DRG (DRG Instruments GmbH, Marburg, Germany). The samples were analyzed blind and cases and controls were randomly mixed in batches using a single lot number of reagent and calibrator. One scientist did all the measurements. Two levels of quality control (QC) material were analyzed with each run on the analyzer and standard Westgard rules applied. Two levels of QC material were included on each plate for the manual ELISA assays. FE2 and fT were calculated using the equation based on the law of mass action (Vermeulen et al. 1999).

Statistical analysis

Mean and median levels of sex steroid hormones, ERα and ERβ and AR SB were calculated for all breast cancer samples and controls. Differences in the medians between the groups were tested for statistical significance using the Kruskal–Wallis test. Correlations between sex steroid hormones, and ERα and ERβ and AR SB among cases and controls were assessed by the Spearman’s rank correlation coefficient. Subjects were classified according to quintiles of the respective marker among controls. The associations between ERα, ERβ, AR SB, hormone levels and the risk of breast cancer were determined by logistic regression analysis controlling for age. Finally, SB levels of each receptor were controlled for all hormones and SB in regression models to estimate their independent associations with breast cancer risk.

Results

The median age of the 200 women with breast cancer (cases) was 61.33 (IQR, 11.32) and 62.33 (IQR, 9.57), in the 400 healthy women (matched controls). Breast tumor characteristics of the cases were similar to a typical breast cancer cohort (Table 1). None of the traditional risk factors (family history, age at menarche, menopause, number of pregnancies, contraceptive pill use, hysterectomy, infertility, body mass index, and height) were significantly different between cases and controls except for fallopian tube ligation (odds ratio (OR) for breast cancer, 0.57; 95% confidence interval (CI), 0.35–0.94; $P=0.029$).

Using all samples, correlations of sex steroid hormones and SHBG with sex steroid receptor SB were investigated. FE2 and fT showed a statistical significant positive correlation and SHBG a negative correlation with ERα, ERβ, and AR SB. All three sex steroid hormone receptor SB correlated with each other (Table 2).

For the purposes of the analysis, women were stratified into groups based on whether their sample was obtained 6 months to $\leq 2$ or $>2–5$ years before breast cancer diagnosis. We decided to use the same cut off as that used in the largest reanalysis by
Key et al. (2002) that included nine prospective studies. For those women who had given a sample >2 years before diagnosis, the serum androgens: androstenedione, testosterone, and fT, and both ERα and ERβ SB showed significant differences between cases and controls (Table 3). We further analyzed the data based on quintiles with subjects being classified according to quintiles of the respective marker among controls. Women with serum ERα bioactivity in the top quintile had a 2.15 (95% CI, 1.05–4.43; P < 0.05)–fold breast cancer risk (Table 4). No association was shown between breast cancer risk and ERβ and AR SB (Table 4). Women with serum levels in the top quintile of androstenedione, testosterone, and fT were significantly associated with 4.36 (95% CI, 1.87–11.55), 2.53 (95% CI, 1.24–5.41), and 2.84 (95% CI, 1.30–6.64)–fold risk for breast cancer respectively (Table 4). Other hormones tested did not show any significant association with breast cancer risk (Table 4). Analysis was also undertaken combining both groups. For the 11 hormones and sex steroid receptor SB, differences between cases and controls were observed for serum androstenedione, testosterone, and fT levels (Table 3). ERα, ERβ, and AR SB did not show any significant association with breast cancer and did not predict risk (Table 4). This observation did not change after adjusting for all hormones and SB. SHBG and serum fT showed significant differences between cases and controls (Table 3). Serum levels in the top quintile of androstenedione, testosterone, fT, and estrone were significantly associated with 2.49 (95% CI, 1.20–5.46), 1.870 (95% CI, 0.97–3.70), 2.02 (95% CI, 0.09–4.24), and 2.21 (95% CI, 1.10–4.59)–fold risk for breast cancer respectively (Table 4). The association of androstenedione, testosterone, and estrone with breast cancer risk remained statistically significant after adjustment for all hormones and SB (data not shown). In addition, women who had serum levels in the top quintile of SHBG had a reduced risk of breast cancer (0.32; 95% CI, 0.13–0.73; P = 0.001; Table 4). Other hormones tested did not show any significant association with breast cancer risk (Table 4).

Discussion

The study adds to the ongoing effort to better understand the association of sex steroid hormones with breast cancer. This report is the first we are aware of that examines the role of sex steroid hormone receptor associated with breast cancer after adjustment for all hormones and AR and borderline significant after adjustment for ERβ for those women who had given a sample >2 years before diagnosis. Furthermore, after adjustment for all hormones and SB both androstenedione and testosterone were independently associated with breast cancer risk (data not shown).

For those women who had given samples ≤2 years before diagnosis, ERα, ERβ, and AR SB did not show any significant association with breast cancer and did not predict risk (Tables 3 and 4). This observation did not change after adjusting for all hormones and SB. SHBG and serum fT showed significant differences between cases and controls (Table 3). Serum levels in the top quintile of androstenedione, testosterone, fT, and estrone were significantly associated with 2.49 (95% CI, 1.20–5.46), 1.870 (95% CI, 0.97–3.70), 2.02 (95% CI, 0.09–4.24), and 2.21 (95% CI, 1.10–4.59)–fold risk for breast cancer respectively (Table 4). The association of androstenedione, testosterone, and estrone with breast cancer risk remained statistically significant after adjustment for all hormones and SB (data not shown). In addition, women who had serum levels in the top quintile of SHBG had a reduced risk of breast cancer (0.32; 95% CI, 0.13–0.73; P = 0.001; Table 4). Other hormones tested did not show any significant association with breast cancer risk (Table 4).

Analysis was also undertaken combining both groups. For the 11 hormones and sex steroid receptor SB, differences between cases and controls were observed for serum androstenedione, testosterone, and fT levels (Table 3). ERα, ERβ, and AR SB did not show any significant association with breast cancer and did not predict risk (Table 4). This observation did not change after adjusting for all hormones and SB. Women who had serum levels in the top quintile of androstenedione, testosterone, and fT had 3.187 (95% CI, 1.74–6.04), 2.15 (95% CI, 1.26–3.71), and 2.35 (95% CI, 1.33–4.26)–fold breast cancer risk respectively (Table 4). The association of androstenedione and testosterone with breast cancer risk remained statistically significant after adjustment for all hormones and SB (data not shown). Other hormones examined did not show any significant association with breast cancer risk (Table 4).
bioactivity using a yeast-based bioassay and sex steroid hormones using conventional immunoassays before breast cancer diagnosis within a well-defined cohort of women diagnosed with estrogen-sensitive breast cancer and healthy controls. Serum ERα and ERβ were significantly higher in postmenopausal women before diagnosis, with women having a twofold increased breast cancer risk if ERα SB was in the top quintile more than 2 years before diagnosis. Estrogens were not found to be significantly different between cases and controls but women with estrone levels in the top quintile <2 years before diagnosis had a twofold increased breast cancer risk. Testosterone and androstenedione were significantly higher among cases compared with controls and showed a strong association with an almost threefold increased breast cancer risk independent of time to diagnosis. However, this was not reflected in serum AR bioactivity that was not associated with breast cancer.

The strengths of this study are 1) the nested case-control design within a well-defined cohort with prospective identification of breast cancer cases, 2) use of standardized protocol for serum sample collection and storage with protocol adherence confirmed by the lack of any difference in mean hormone or steroid receptor SB levels between the different trial centers (data not shown), 3) confirmation of breast cancer diagnosis and receptor status from the treating physicians that eliminated possible misidentification of cases from use of cancer registry data or self-reporting alone, 4) well-defined homogenous cases through use of strict eligibility criteria (women not on HRT with ER-positive invasive breast cancer), and 5) selection of controls from the same population as those with breast cancer.

Our observations that ERα and ERβ SB were significantly higher in postmenopausal women before diagnosis of invasive ER-positive breast cancer extend our previous findings of elevated bioactivity in women with breast cancer at the time of clinical diagnosis (Widschwendter et al. 2009). The receptor SB showed statistically significant correlation with fE2 that has the highest known affinity for ERα (Lippman et al. 1977). This is in keeping with the meta-analysis results that women with high E2 levels more than 2 years before diagnosis had a higher breast cancer risk compared with those who had high E2 levels closer to diagnosis (Key et al. 2002). Serum receptor activation is probably modulated by other surrogates as well. In our previous study, receptor SB was two- to threefold higher than the actual E2 concentration (Widschwendter et al. 2009). This may explain the increased breast cancer risk in

Table 2 Spearman’s correlation coefficients among estrogens, androgens, SHBG, and serum bioactivity of estrogen and androgen receptors for cases and controls combined

<table>
<thead>
<tr>
<th></th>
<th>ERα</th>
<th>ERβ</th>
<th>AR</th>
<th>Body mass index</th>
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<tbody>
<tr>
<td>Estradiol (E2)</td>
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<td>0.062</td>
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<td>573</td>
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<td>Free E2</td>
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<td>0.045</td>
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Table 3 Comparison of sex steroid hormones and sex steroid receptor serum bioactivity levels 6 months to 5 years before breast cancer diagnosis between cases and controls

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<th>Hormones and serum bioactivity</th>
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</table>

AR, androgen receptor; DHEAS, dehydroepiandrosterone sulfate; ER, estrogen receptor; SHBG, sex hormone-binding globulin; STD, standard deviation.

*Numbers do not always add up to 200 cases and 400 controls due to some missing values.
*Kruskal–Wallis for difference in median value between cases and controls.

AR, androgen receptor; DHEAS, dehydroepiandrosterone sulfate; ER, estrogen receptor; SHBG, sex hormone-binding globulin; STD, standard deviation.

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AR, androgen receptor; DHEAS, dehydroepiandrosterone sulfate; ER, estrogen receptor; SHBG, sex hormone-binding globulin; STD, standard deviation.
Table 4 Sex steroid receptor serum bioactivity (A) sex steroid hormones (B) and breast cancer risk

<table>
<thead>
<tr>
<th>Quintile Range</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Quintile Range</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Quintile Range</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st 0–27.06</td>
<td>1.00 (ref.)</td>
<td>77</td>
<td>14</td>
<td>1.00 (ref.)</td>
<td>77</td>
<td>22</td>
<td>1.00 (ref.)</td>
<td>77</td>
</tr>
<tr>
<td>2nd 27.06–52.73</td>
<td>1.22 (0.56–2.68)</td>
<td>76</td>
<td>17</td>
<td>1.08 (0.48–2.41)</td>
<td>76</td>
<td>16</td>
<td>0.74 (0.36–1.50)</td>
<td>76</td>
</tr>
<tr>
<td>3rd 52.73–70.01</td>
<td>1.49 (0.70–3.23)</td>
<td>76</td>
<td>20</td>
<td>1.97 (0.49–1.62)</td>
<td>76</td>
<td>21</td>
<td>1.01 (0.46–1.81)</td>
<td>76</td>
</tr>
<tr>
<td>4th 70.01–104.36</td>
<td>2.11 (1.05–4.43)</td>
<td>76</td>
<td>29</td>
<td>1.01 (0.46–1.81)</td>
<td>76</td>
<td>21</td>
<td>1.01 (0.46–1.81)</td>
<td>76</td>
</tr>
<tr>
<td>5th 104.36–459.22</td>
<td>2.11 (1.05–4.43)</td>
<td>76</td>
<td>29</td>
<td>1.01 (0.46–1.81)</td>
<td>76</td>
<td>21</td>
<td>1.01 (0.46–1.81)</td>
<td>76</td>
</tr>
</tbody>
</table>

(A) Serum bioactivity
ERα (pg/ml) 1st 0–27.06 77 14 1.00 (ref.)
2nd 27.06–52.73 76 17 1.22 (0.56–2.68)
3rd 52.73–70.01 76 15 1.08 (0.48–2.41)
4th 70.01–104.36 76 20 1.49 (0.70–3.23)
5th 104.36–459.22 76 29 2.11 (1.05–4.43)

ERβ (pg/ml) 1st 0–2.97 77 13 1.00 (ref.)
2nd 2.97–34.36 76 13 0.98 (0.42–2.23)
3rd 34.36–55.80 76 18 1.41 (0.65–3.13)
4th 55.80–98.96 76 25 1.95 (0.94–4.20)
5th 98.96–477.56 77 29 2.11 (1.05–4.43)

AR (pg/ml) 1st 0.36–1.59 77 17 1.00 (ref.)
2nd 1.59–2.10 76 10 0.60 (0.25–1.37)
3rd 2.10–2.45 76 23 1.38 (0.68–2.81)
4th 2.45–2.87 76 21 1.26 (0.62–2.59)
5th 2.87–7.45 76 23 1.38 (0.68–2.82)

(B) Hormone
Estradiol (E2; pg/ml) 1st 0–11.47 76 16 1.00 (ref.)
2nd 11.47–14.74 75 13 0.84 (0.37–1.88)
3rd 14.74–17.98 75 22 1.39 (0.68–2.90)
4th 17.98–22.66 75 20 1.27 (0.61–2.67)
5th 22.66–209.40 75 23 1.46 (0.72–3.03)

Free E2 (pg/ml) 1st 0–0.50 76 14 1.00 (ref.)
2nd 0.50–0.69 75 15 1.07 (0.48–2.40)
3rd 0.69–0.90 75 27 1.95 (0.96–4.10)
4th 0.90–1.19 75 15 1.10 (0.49–2.46)
5th 1.19–6.62 75 23 1.66 (0.80–3.54)

Estrone (pg/ml) 1st 0–56.28 76 14 1.00 (ref.)
2nd 56.28–72.63 75 23 1.65 (0.78–3.52)
3rd 72.63–90.14 75 22 1.58 (0.76–3.39)
4th 90.14–115.53 75 15 1.07 (0.48–2.43)
5th 115.53–779.83 75 21 1.53 (0.73–3.31)
Table 4 continued

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Range</th>
<th>Controls</th>
<th>More than 2 years</th>
<th>Controls</th>
<th>&lt;2 years</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>n</td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Androstenedione (ng/dl)</td>
<td>1st 0–52.44</td>
<td>76 7 1.00 (ref.)</td>
<td>76 12 1.00 (ref.)</td>
<td>76 19 1.00 (ref.)</td>
<td>2nd 52.44–77.94</td>
<td>76 18 3.09 (1.23–8.65)</td>
<td>0.022 76 23 2.18 (1.01–4.95) 0.054 76 41 2.52 (1.33–4.95) 0.006 3rd 77.94–102.87</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>1st 0–0.16</td>
<td>77 13 1.00 (ref.)</td>
<td>77 18 1.00 (ref.)</td>
<td>77 31 1.00 (ref.)</td>
<td>2nd 0.16–0.22</td>
<td>75 15 1.22 (0.54–2.80)</td>
<td>0.636 75 19 1.15 (0.55–2.39) 0.714 75 34 1.16 (0.65–2.10) 0.613 3rd 0.22–0.38</td>
</tr>
<tr>
<td>Free testosterone (ng/dl)</td>
<td>1st 0–0.05</td>
<td>79 10 1.00 (ref.)</td>
<td>79 14 1.00 (ref.)</td>
<td>79 24 1.00 (ref.)</td>
<td>2nd 0.05–0.08</td>
<td>76 15 1.93 (0.84–4.62)</td>
<td>0.126 76 24 1.81 (0.88–3.66) 0.112 76 42 1.85 (1.03–3.38) 0.044 3rd 0.08–0.11</td>
</tr>
<tr>
<td>DHEAS (µg/ml)</td>
<td>1st 0–58.44</td>
<td>77 17 1.00 (ref.)</td>
<td>77 16 1.00 (ref.)</td>
<td>77 33 1.00 (ref.)</td>
<td>2nd 58.44–85.52</td>
<td>77 22 1.29 (0.64–2.66)</td>
<td>0.483 77 15 0.96 (0.44–2.09) 0.92 77 37 1.14 (0.65–2.02) 0.654 3rd 85.52–119.16</td>
</tr>
<tr>
<td>SHBG (µg/ml)</td>
<td>1st 0–346.53</td>
<td>77 15 1.00 (ref.)</td>
<td>77 25 1.00 (ref.)</td>
<td>77 40 1.00 (ref.)</td>
<td>2nd 346.15–485.79</td>
<td>77 30 1.99 (1.00–4.09)</td>
<td>0.053 77 20 0.81 (0.41–1.57) 0.53 77 50 1.24 (0.73–2.10) 0.418 3rd 485.79–634.32</td>
</tr>
</tbody>
</table>

OR values for quintiles based on controls only being age adjusted. OR with P values ≤0.05 marked with bold. AR, androgen receptor; DHEAS, dehydroepiandrosterone sulfate; ER, estrogen receptor; OR, odds ratio; SHBG, sex hormone-binding globulin.
unable to demonstrate such an association. While fT is the best ligand of AR, androgens have also been shown to bind and activate ERs (Maggioni et al. 1999a). Our data showing a statistically significant correlation between fT and both ERs favor the view for the existence of the latter pathway where androgens promote breast cell proliferation by binding directly to ER.

To summarize, our findings provide further evidence of the association between sex steroid hormones and breast cancer risk. Testosterone and estrone were shown to be associated with increased breast cancer risk. Based on that, it would be interesting to evaluate the association of key enzymes in steroidogenesis such as aromatase and 17β-hydroxysteroid dehydrogenases and breast cancer. In addition, our report provides novel insight into the role of sex steroid receptor SB in breast cancer with ER but not AR SB associated with increased risk more than 2 years before diagnosis. Further development of these assays might appear promising for giving greater insight into the role of sex hormones in relation to breast cancer risk but on the basis of the current results the assays do not appear to have a stronger association with breast cancer risk compared with this and previous studies using conventional assays. If ER SB results are validated in other studies, it may also prove beneficial in individualizing and monitoring breast cancer chemopreventive strategies using antiestrogens such as tamoxifen (Cuzick et al., 2003), raloxifene (Fabian & Kimler 2005), and aromatase inhibitors (Kalidas & Brown 2005).

Declaration of interest

I Jacobs has consultancy arrangements with Becton Dickinson, who have an interest in tumor markers and ovarian cancer. They have provided consulting fees, funds for research, and staff but are not directly related to this study. U Menon has a financial interest through UCL Business and Abcodia Ltd. in the third party exploitation of clinical trials biobanks, which have been developed through the research at UCL. No other financial disclosures.

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References


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