Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

04.05.2011

Postmenopausal serum sex steroids and risk of hormone receptor positive and negative breast cancer: a nested case-control study

Rebecca E James[1], Annekatrin Lukanova[1], Laure Dossus[1], Susen Becker[2], Sabina Rinaldi[3], Anne Tjønneland[4], Anja Olsen[4] Kim Overvad[5], Sylvie Mesrine[6 7], Pierre Engel[6 7], Françoise Clavel-Chapelon[6 7], Jenny Chang-Claude[1], Alina Vrieling[1], Heiner Boeing[8], Madlen Schütze[8], Antonia Trichopoulou[9 10], Pagona Lagiou [9 11], Dimitrios Trichopoulos D[11 12], Domenico Palli[13], Vittorio Krogh[14], Salvatore Panico[15], Rosario Tumino[16], Carlotta Sacerdote[17], Laudina Rodríguez[18], Genevieve Buckland[19] Maria-José Sánchez[20], Pilar Amiano[21], Eva Ardanaz[22 23], Bas Bueno-de-Mesquita[24], Martine M Ros[24] , Carla H van Gils [25], Petra H Peeters[25], Kay-Tee Khaw[26], Nick Wareham[27], Timothy J Key[28], Naomi E Allen[28], Isabelle Romieu[29], Afshan Siddiq[30], David Cox[30], Elio Riboli[31], Rudolf Kaaks [1]

Running Title: Postmenopausal sex steroids and breast cancer by receptor status.

Key Words: estradiol, testosterone, sex hormone binding globulin, postmenopausal breast cancer, receptor status

Word count: *233 (*abstract) 2369 *(t*ext) (1662 methods)

[1]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.
[2] Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Universitätsklinikum Leipzig
[3] Biomarkers Group, Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC), Lyon, France
[4] Institute of Cancer Epidemiology, Danish Cancer Society, 2100 Copenhagen, Denmark
[5] Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark
[6] Inserm, Centre for Research in Epidemiology and Population Health, U1018, Institut Gustave Roussy, F-94805, Villejuif, France
[7] Paris South University, UMRS 1018, F-94805, Villejuif, France
[8] Department of Epidemiology, German Institute of Human, Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[9] WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, 75 M. Asias Avenue, Goudi, GR-115 27, Athens, Greece
[10] Hellenic Health Foundation, 10-12 Tetrapoleos Street, GR-115 27, Athens Greece
[11] Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA and
[12] Bureau of Epidemiologic Research, Academy of Athens, 28 Panepistimiou Street, Athens, GR-106 79, Greece
[13] Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute - ISPO, Florence Italy
[14] Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale Tumori
[15] Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy.
[16] Cancer Registry and Histopathology Unit, "Civile M.P.Arezzo" Hospital, ASP 7 Ragusa (Italy)
[17] HuGeF Foundation and CPO-Piemonte, Torino, Italy, Torino Italy.
[18] Public Health and Participation Directorate, Health and Health Care Services Council, Asturias, Spain.
[19] Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO-IDIBELL), Spain
[20] Andalusian School of Public Health, Granada (Spain) and CIBER de Epidemiología y Salud Pública (CIBERESP), Spain.
[21] Public Health Division of Gipuzkoa, Instituto Investigación BioDonostia, Basque Government; CIBER Epidemiología y Salud Pública, CIBERESP, Spain.
[22] Navarre Public Health Institute, Pamplona, Spain.
[23] Consortium for Biomedical Research in Epidemiology and Public Health (CIBER Epidemiología y Salud Pública-CIBERESP), Spain.
[24] National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[25] Julius Center for Health Sciences and Primary Care, University Medical Center, 3508 GA Utrecht, The Netherlands
[26] University of Cambridge
[27] MRC Epidemiology Unit, Cambridge
[28] Cancer Epidemiology Unit, University of Oxford, Oxford, UK
[29] Nutritional Epidemiology Group, Section of Nutrition and Metabolism, IARC
[30] Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London
[31] School of Public Health, Imperial College London

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

ABSTRACT

Background: Pre-diagnostic endogenous sex steroid hormone levels have well established associations with overall risk of breast cancer. While evidence towards the existence of distinct subtypes of breast cancer accumulates, few studies have investigated the associations of sex steroid hormone levels with risk of hormone receptor (estrogen (ER) and/or progesterone receptor (PR)) defined breast cancer.

Methods: In a case-control study nested within the EPIC cohort (European Prospective Investigation into Cancer and Nutrition), estradiol, testosterone and sex hormone binding globulin levels were measured in pre-diagnostic serum samples from postmenopausal women not using HRT at blood donation. 554 women who developed invasive breast cancer with information on receptor status were matched with 821 control subjects. Conditional logistic regression models estimated breast cancer risk with hormone concentrations according to hormone receptor status of the tumor.

Results: Sex steroid hormones were associated with risks of not only ER+PR+ breast cancer [estradiol OR for highest versus lowest tertile=2.91 (95%CI:1.62-5.23), P_{trend}0.002; testosterone OR=2.27 (95%CI:1.35-3.81), P_{trend}=0.002] but also of ER-PR- breast cancer [estradiol OR=2.11 (95%CI:1.00-4.46), P_{trend} =0.05; testosterone OR= 2.06 (95%CI:0.95-4.46), P_{trend}=0.03], with associations appearing somewhat stronger in the receptor positive disease.

Conclusions: Serum androgens and estrogens are associated with risks of both hormone receptor negative as well as receptor positive breast tumors. Further

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

research is needed to establish through which molecular pathways, and during which evolutionary stages of development, androgens and estrogens can promote the occurrence of both receptor-positive and –negative clinical breast tumors.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Introduction

Breast cancer is a complex and heterogeneous disease, with a variety of histo-pathological and molecular sub-forms that have diverse clinical outcomes and risk factors (1;2). One important classification of clinical breast tumors into subtypes is based on the presence or absence of estrogen receptor (ER) and progesterone receptor (PR), as well as HER2 receptors, and the routine identification of these receptors currently guides targeted therapies and provides important prognostic information (2;3). The presence or absence of hormone receptors also broadly corresponds to more detailed molecular sub-classification of breast tumors, as determined by microarray-based gene expression profiling coupled to hierarchical clustering analyses (4-6). ER-positive disease accounts for about 80% and PR-positive for about 65% of breast cancer cases (7;8).

Epidemiologic studies have demonstrated that risk factors associated with increased lifetime exposures to estrogen such as early age at menarche, late age at menopause, postmenopausal hormone replacement therapy and postmenopausal adiposity levels are associated with hormone receptor positive breast cancer particularly, more than with risk of ER-negative tumors (2). Although the associations of endogenous sex hormone levels with the risk of breast cancer overall, all subtypes combined, are well established (9;10), only few prospective studies have thus far investigated this association by hormone receptor status (2;11-15). In the latter studies, estradiol and testosterone levels have shown direct relationships with ER-positive, PR-positive, and joint ER+PR+ tumors (14;15). Meanwhile, prospective studies investigating associations with

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

hormone receptor negative or HER2-positive breast cancer have been limited by relatively small numbers of ER-negative tumors(14;15).

Previously, the relationship between postmenopausal sex steroid hormone and SHBG levels with breast cancer risk overall was investigated in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (10). The current analysis expands on this nested case-control study with additional breast cancer cases, with a particular focus on receptor status and with over-sampling of ER-negative cases. To our knowledge, this is the largest prospective study to date to investigate the association of estradiol, testosterone, their non-sex hormone binding globulin (SHBG) bound fractions, and SHBG with the risk of breast cancer by hormone receptor status, with a total of 172 ER-negative and 382 ER-positive cases.

Materials and Methods

EPIC is a multi-center prospective cohort study designed to investigate the relationships between diet, nutrition and metabolic factors and cancer, consisting of 366 521 women and 153 457 men aged mostly between 25-70 years(16;17). All participants were enrolled between 1992 and 2000 from 23 centers in 10 European countries: Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, The Netherlands and the United Kingdom.

Baseline anthropometric measurements and questionnaire data on habitual diet, reproductive and menstrual history, exogenous hormone use [oral contraceptive (OC) and hormone replacement therapy (HRT) use], medical history, lifetime smoking and alcohol consumption history, occupation, level of

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

education and physical activity were collected. Blood samples were also collected for most participants. Details about the standardised procedures for recruitment, measuring baseline anthropometry (height, weight, waist and hip circumferences), questionnaires and biological sample collection at study centers are given elsewhere (16;17). Sweden and Norway were not included in the current analysis because independent studies were being completed on breast cancer risk, or because a blood serum sample was not available. All subjects gave written informed consent to use their questionnaire data and future analyses of their blood samples. The Internal Review Boards (IRB) of IARC and all EPIC recruitment centers approved the analyses of serum samples.

Blood samples were collected according to standardized protocols. From each subject, 30mL of blood was drawn and after centrifugation, blood fractions (serum, plasma, buffy coat, and red blood cells) were aliquoted in 28 plastic straws of 0.5mL each (12 plasma, 8 serum, 4 erythrocytes and 4 buffy coat for DNA), which were heat-sealed and stored under liquid nitrogen (-196°C). Half of the 28 aliquots were stored locally and the other half centrally at the International Agency for Research on Cancer (IARC), with the exception of Denmark blood samples were stored locally in 1mL tubes at -150°C.

In all countries (except for France, Greece and Germany) incident breast cancer cases were identified using a combination of methods employing record linkage with cancer and pathology registries. Vital status was collected from regional or national mortality registries. In Greece, Germany and France, active follow up of cancer was through health insurance records and direct contact of participants and their next of kin was used. Self reported breast cancer cases

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

were all systematically verified from clinical and pathological records. The closure date for this follow up period was the date of last complete follow up for both cancer incidence and vital status, which ranged between 2003 and 2006, depending on the center. Cancer incidence data were classified according to International Classification of Diseases 10th Revision (ICD-10).

Information on participants' receptor status, as well as the available laboratory methods and quantification descriptions used to determine receptor status was collected by 20 centers using the same techniques to collect incident breast cancer cases. At the time of this study, insufficient information on Her2 status (n=37) had been collected to be included in the current analysis. To standardize the quantification of receptor status received from the EPIC centers, the following criteria for a positive receptor status were used; ≥10% cells stained, any 'plus-system' description, ≥20 fmol/mg, an Allred score of ≥3, an IRS ≥2, or an H-score ≥10 (18-22).

The present analysis was based upon postmenopausal female participants with a blood sample, after *a priori* excluding women with prevalent cancer at any organ site (except non melanoma skin cancer) at baseline examination. Women were considered postmenopausal at the time of blood collection if they had had no menstrual cycles in the last 12 months, were older than 55 years (if the menstrual cycle history was missing), or had reported a bilateral oophorectomy. All women included in this analysis were not using HRT at the time of blood donation.

This study expands on a previous case-control study nested within EPIC on postmenopausal breast cancer risk and endogenous hormone

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

concentrations(10). Additional cases were women who subsequently developed breast cancer after blood donation and before the end of the study period. For each case subject, up to two control subjects with a blood sample were chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer at the time of diagnosis of the index case. An incidence density sampling protocol was used, such that controls could include subjects who became a case later in time, while each control could be sampled more than once. The matching criteria were the study recruitment center, age at blood donation (+/- 6 months), time of the day of blood collection (+/- 1 hour), and fasting status (<3 hours, 3-6 hours, >6 hours).

Among the cases included in the study conducted in 2004(10), only 49% had information on hormone receptors and were included in the present analysis (329 cases plus 596 matched controls)(10); this part of our previous study is now referred to as 'Study phase 1'. After completing a new round of follow-up in EPIC, all newly identified ER-negative cases, plus an equal number of ER-positive cases along with their matched controls were included (225 cases and 225 controls; this is now referred to as 'Study phase 2'). Overall, a total of 554 (382 ER-positive and 172 ER-negative) cases and 821 matched controls were thus included in this analysis.

Hormone assays in study phase 1 were performed at IARC, while in study phase 2 hormone assays were performed at the German Cancer Research Center (DKFZ), using the same assays whenever possible.

In study phase 1, estradiol concentrations were determined using a radioimmunoassay with a double-antibody system for the separation of free and

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

bound antigen (Diagnostic Systems Laboratories Inc. (DSL), Webster, TX, USA). Because this assay was no longer produced by the company when study phase 2 started, estradiol was assayed using a similar double-antibody radioimmunoassay (DiaSorin, Saluggia, Italy). In both study phases, testosterone concentrations were measured with the same radioimmunoassay (Immunotech, Marseille, France). A solid phase “sandwich” immunoradiometric assay (Cis-Bio International, Gif-sur-Yvette, France) was used for the analysis of SHBG levels.

Serum samples were thawed once and all hormone measurements performed on one day to avoid additional freeze-thaw cycles. Laboratory technicians were blinded to the case-control status of the study subjects. Cases and their individually matched controls were always analyzed within the same analytical batch.

Mean intra-batch coefficients of variation were 5.8% and 11.4% for estradiol, 10.8% and 8.2% for testosterone and 8.0% and 3.1% for SHBG for study phases 1 and 2 respectively. Inter-batch coefficients of variation were 13.1% and 13.4% for estradiol, 15.3% and 14.0% for testosterone and 16.5% and 7.1% for SHBG for study phases 1 and 2 respectively. Serum concentrations of free testosterone and free estradiol (unbound to SHBG or albumin) were calculated from mass action equations using absolute concentrations of each steroid and SHBG and assuming a constant serum albumin concentration of 43g/L (23;24).

In all analyses, levels of estradiol (pmol/L), free estradiol (pmol/L), testosterone (nmol/L), free testosterone (pmol/L) and SHBG (nmol/L) were log2-transformed to normalize their variable distributions. Statistical significance of

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

baseline case-control differences was evaluated using conditional logistic regression. Correlations between hormones and anthropometric indices, adjusting for age at blood donation and laboratory batch were calculated using Pearson's correlation coefficient. Statistical significance of case-control differences in geometric mean hormone levels were evaluated using paired t-tests of case values versus the average of the two matched controls.

Conditional logistic regression models were used to estimate odds ratios and 95% confidence intervals for breast cancer sub-classified by single hormone receptor status (ER-positive/negative or PR-positive/negative) or joint hormone receptor status (ER+PR+, ER+PR-, ER-PR-) at different serum hormone concentrations. There were too few breast cancer cases with the joint ER-PR+ receptor pattern (n=11) to be considered as a separate outcome category. The risk associated with serum levels of the sex steroid hormones was examined both on the log2 continuous scale and in tertiles.

To statistically account for the differences observed between study phase 1 and study phase 2, tertile cut-points were based on the study phase-specific hormone distributions in control subjects.

Likelihood ratio tests were used to assess linear trends in odds-ratios (ORs) with increasing exposure level with assigned quantitative scores 1, 2 and 3 for the tertile categories. Heterogeneity between breast cancer subtypes was assessed using a log likelihood ratio test to assess conditional logistic regression models with and without interaction terms for breast cancer subtype outcome (ER-positive ER-negative, PR-positive, PR-negative or ER+PR+, ER+PR-, ER-

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

PR-). Interaction terms were created by multiplying breast cancer subtype with the linear trend over the tertile score of hormone levels.

The effects of potential confounders (other than those accounted for by the matching criteria) were examined by adding regression terms into the logistic regression models. The categorical variables included age at menarche (less than 12 years, 12 years, 13 years, 14 years and greater than 14 years), age at first childbirth (nulliparous, less than 23 years, 24-25 years, 26-28 years, greater than 29 years), number of full-term births (nulliparous, 1 full-term birth, 2 full-term births , 3 full-term births, 4 or more full-term births), history of breastfeeding (ever versus never), oral contraceptive (OC) (ever versus never), hormone therapy use (ever versus never), age at menopause, smoking status (current, former never), alcohol consumption (less than 1.5g/day, 1.5g-10g/day, 10-20g/day and greater than 20 g/day), physical activity (active, moderately active, moderately inactive and inactive) (25), education level (none, primary school, technical/professional school, secondary school, longer education including university degree or not specified) and BMI (continuous). Missing values (generally <2%) were accounted for by creating an extra category in each covariate.

To assess differences in risk estimates by subgroups, analyses based on the continuous log2 scale were stratified by study phase, time between blood donation and diagnosis less than and greater than 2 years, age at diagnosis (greater than/less than 64 years) and BMI above and below the median. Analyses by subgroups of BMI were performed using unconditional logistic regression adjusting for matching factors. Sensitivity analyses excluding past

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

users of OC or HRT were also performed. Heterogeneity between the subgroups was assessed using Cochrane's Q-statistic (26).

Results

The mean age at blood donation was 59.8 and 60.1 years, for cases and controls respectively (**Table 1)**. At the time of blood donation, the mean time since menopause was 10.7 years for cases and 11.1 years for age-matched controls. The mean years since menopause to breast cancer diagnosis was 14.4 years in study phase 1 and 15.9 in study phase 2. There was a mean time lag of 4.3 years after blood donation until breast cancer diagnosis. Compared to control subjects, cases had a higher body-mass-index (BMI) (26.4 versus 26.0, p=0.02). Due to the study design, 112 (50%) of the 225 breast cancer cases from study phase 2 were ER-negative.

Generally, in both study phases, geometric mean levels of all sex steroids were significantly higher among cases compared to controls (**Table 2)**. Differences in absolute estrogen levels were observed between the two study phases, which may be explained by the different radioimmunoassays used to measure estradiol, as indicated also by measurements on quality control samples. SHBG mean levels were only lower in cases than in controls in study phase 2. Likewise, differences in absolute SHBG levels observed between the study phases 1 and 2 may be explained by changes over time in antibodies, standard curves and standards of the assay kit used for this study (Cis-Bio International, Gif-sur-Yvette, France).

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Correlations between all sex steroids and SHBG levels adjusted for batch and age at blood donation were similar in magnitude in both study phases with the exception of weaker correlations observed in study phase 2 for testosterone with estradiol, and of free testosterone with free estradiol (**Supplementary Table 1).**

In the conditional logistic regression analyses, levels of total estradiol showed direct associations with risks of both ER-positive breast cancer (OR for upper *versus* lower tertiles: 2.58 [95%CI: 1.69-3.93] P_{trend} <0.0001) and ER-negative breast cancer subtypes (OR: 1.65 [95%CI: 0.91-2.98] P_{trend} =0.09) with associations showing no significant heterogeneity (P_{het}=0.88) (**Figure 1**). Likewise, total testosterone also showed direct associations with risks of both ER-positive (OR: 1.68 [95%CI: 1.16-2.44] P_{trend} =0.006) and ER-negative breast cancer subtypes (OR: 1.75 [95%CI: 0.94-3.35] P_{trend} =0.04), which also did not show significant heterogeneity (P_{het}=0.99). There was a significant negative association of SHBG with the risk of ER-positive breast cancer (OR: 0.71 [95%CI: 0.51-1.00] P_{trend} =0.04) but not with ER-negative cancer (OR 0.73 [95%CI: 0.43-1.25] P_{trend} =0.25), however this was not heterogeneous (P_{het}=0.98). A similar degree of heterogeneity between breast cancer subtypes was seen when defined by PR status **(Supplementary figure 1).**

Further differentiation of the ER-positive tumors by including PR receptor information showed the strongest direct risk associations among all subtypes with ER+PR+ cancer (estradiol OR: 2.91 [95%CI: 1.62-5.23], P_{trend} 0.0002; testosterone OR: 2.27 [95%CI: 1.35-3.81] P_{trend} =0.002) (**Figure 2**). Interestingly, however, stronger observations were also seen in the ER-PR- subtypes

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

[estradiol OR: 2.11 (95%CI: 1.00-4.46) P_{trend} = 0.05; testosterone OR: 2.06 (95%CI: 0.95-4.46) P_{trend} =0.03] in comparison to the single receptor ER-negative defined breast cancer odds ratios. Relative risk estimates for hormone levels with the discordant ER+/PR- receptor breast cancer subtype demonstrated no distinct associations. Similar risk estimates of SHBG were observed with joint ER+PR+ breast cancer (OR: 0.74 [95%CI: 0.46-1.21] P_{trend} =0.22) and ER-PR- tumors (OR 0.89 [95%CI: 0.48-1.68] P_{trend} =0.73, P_{het}=0.34).

With the exception of SHBG, relative risk estimates did not change after adjustment for BMI (data not shown). After adjusting for BMI, the significant negative risk association of SHBG with ER-positive disease was no longer significant [OR: 0.75 (95%CI: 0.52-1.08) P_{trend} =0.10]. Risk estimates for estradiol and testosterone were stronger in ER-positive tumors after adjusting for age at menopause and age at first childbirth [estradiol OR: 2.69 (95%CI: 1.73-4.20) P_{trend} <0.0001 and testosterone OR: 1.78 (95%CI: 1.22-2.62) P_{trend} =0.003]. Risk associations of all hormones with ER-negative breast tumors were slightly attenuated after adjusting for age at menopause and age at first childbirth [estradiol OR: 1.26 (95%CI: 0.65-2.44) P_{trend} =0.44 and testosterone OR: 1.65 (95%CI: 0.85-3.21) P_{trend} =0.10].

Associations for estradiol and testosterone with the risk of ER-positive breast cancer did not remarkably change after excluding women who were diagnosed within 2 years of blood donation (**Supplementary Figure 1).** Restricting analyses to women diagnosed within two years of blood donation, significant risk associations were no longer observed for total estradiol and testosterone for the ER-positive breast cancer. ER-negative breast cancer risk

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

estimates in total estradiol and testosterone did not differ between women diagnosed before and after two years of blood donation. For women diagnosed within two years of blood donation, SHBG concentrations on a continuous log2 scale showed an unexpected risk association with ER-positive breast cancer (OR: 1.75 [95%CI: 1.13-2.742]), statistically heterogeneous from the association seen for breast cancer diagnosed after 2 years (P_{het}=0.0004). For study phase1, a maximum follow-up time of 13.2 years (median 8.3 years) was observed, thus extending well beyond the 2-year cut point. A similar heterogeneity of circulating sex hormones by study phase and duration of follow-up greater or less than 2 years was also seen for the two study phases combined.

Relative risk estimates were of similar magnitude when breast cancer cases were stratified into those diagnosed before age 64 (the median age at diagnosis) and after 64 years (data not shown). Furthermore, there was no clear heterogeneity between relative risk estimates in the subgroups of women with a BMI below and above the median (25.5kg/m^2). Excluding women who had reported previous use of HRT or OC did not affect the associations in each subtype.

Discussion

Prospective cohort studies, including our earlier study in the EPIC cohort, have shown an increased risk of breast cancer risk overall with increases in postmenopausal blood concentrations of estrogens and androgens (10). Our present, extended analysis within the EPIC cohort shows that serum levels of total and bioavailable testosterone and estradiol are associated with risks of ER-

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

positive, PR-positive and joint ER+PR+, as well as of ER-negative, PR-negative and joint ER-PR- breast tumors.

To our knowledge, six previous prospective studies have so far reported the risk association of sex steroid hormone levels with breast cancer risk by receptor status, and they are summarized in **Table 4**. As can be seen from this table, these studies have generally shown direct risk associations with estrogen and/or testosterone with ER-positive, PR-positive and joint ER+PR+ breast cancer subtypes (27). However, due to the small numbers of hormone receptor negative cases, each of these previous studies had little power to find significant associations within the ER-negative, PR-negative or joint ER-PR- breast cancer subtypes. Our present study, although itself of limited size, includes substantially more ER-negative cases of breast cancer than most previous prospective studies, and shows significant direct associations of both serum estradiol and testosterone with ER-negative, PR-negative and joint ER-PR-negative breast cancer.

While receptor status determination of breast tumors has become a standard part of breast cancer diagnosis, there is variation between laboratory methods and quantification systems for determining receptor status, and this was reflected also across the EPIC centers. Nevertheless, a number of studies have shown that the classification of receptors is relatively robust and have only moderate dependence on the assay used especially for medium and high-expressing tumors (28). With respect to our hormone measurements, we observed some important differences in the absolute scales of measurement between phases 1 and 2 of our study, most likely due to changes in the

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

(commercial) assays used. However, cases and controls were matched by laboratory batch, and further standardization across the two phases was achieved by using phase-specific quartile cut-points for the estimation of odds ratios of breast cancer.

Whether estrogens promote the early development of breast tumors or whether increased circulating estrogens mostly promote late-stage growth of an established tumor, or both, is still unclear (11;29). Observations from studies relating breast cancer risk and population incidence rates with the use of postmenopausal hormone (estrogen alone, or estrogen plus progestin) therapy suggest that effects of estrogens on breast tumor development could be essentially late-stage (29;30). In our study, interestingly, serum estradiol concentrations were associated more strongly with risk of breast cancer diagnosed at least two years after blood donation than for breast cancer diagnosed within a less than a two-year interval. These latter observations would thus seem to argue against the concept that estrogens play a tumor promoting role especially in the very last stages. Other studies, however, did not report the same heterogeneity of association of serum endogenous estrogens with breast cancer risk by lag-time between blood donation and breast tumor diagnosis (1;31). Furthermore, the negative risk association with increasing SHBG, and its inverse relationship with bioavailable estradiol and testosterone could be an artifact due to inverse causation. For example, weight loss relatively shortly before breast cancer diagnosis could have led to an increase in SHBG levels, and to decreases in bioavailable (non-SHBG bound) estradiol and testosterone.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Estrogens are believed to play key roles in the development of normal breast tissue, as well as in breast cancer progression (32;33). Estrogen binding to ER-alpha results in the stimulation of cell proliferation and inhibition of apoptosis, thereby stimulating tumor growth (34;35), and clinical studies have shown that, indeed, only patients with ER-positive tumors respond favorably to anti-estrogenic adjuvant therapy (3;36). It thus seems paradoxical that, in our analysis, estrogens do also show a direct association with ER-negative, PR-negative and joint ER-PR- breast cancer, with an effect size that was only moderately smaller than that seen for hormone receptor positive disease. A direct, but statistically non-significant, association for estrogens with hormone receptor negative breast cancer was also observed in two previous prospective cohort studies (11;13) and, taken together, these various observations could be interpreted as to suggest that estrogens act through molecular pathways that do not directly involve the ER-alpha receptor. However, breast cancer development is a slow process that evolves through several evolutionary stages. Estrogens may not only have late-stage effects on growth promotion of tumors (especially of ER-positive type) but probably also play an important role in earlier evolutionary stages of development, and indeed, available evidence suggests that most ER-negative ductal carcinomas *in situ* or invasive breast cancers arise from ER-positive precursors or cells that stop expressing the receptor (37).

The specific role that androgens may have in breast cancer etiology has long been debated (33;38). One concept is that androgens (androstenedione and testosterone), convert into estrogens (estrone and estradiol, respectively) thus stimulating the growth and division of breast cells (33). Estradiol concentrations

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

are higher in tumors than in non-malignant tissue, suggesting increased aromatase activity in mammary cancers and (39;40), possibly further increasing breast cancer risk through local estrogen synthesis. This local conversion of androgens into estrogens within breast tissue is likely to be a relevant physiologic mechanism especially with respect to the development of ER-responsive tumors which, as discussed above, may also include some fraction of tumors that do not express ER-alpha any longer at the time of their clinical manifestation. Almost all ER-positive tumors are also androgen receptor (AR) positive (40), however, and the same is true for a considerable proportion of ER-negative breast carcinomas(41). Furthermore, the ER-negative/PR-negative/AR-positive breast tumor subtype has been further indicated as being androgen dependent in its growth (42). These observations suggest that large fractions of both ER-positive and ER-negative tumors could also respond directly to the growth promoting effects of testosterone, through pathways that may not necessarily depend on the estrogen alpha receptor. A further interesting observation, in this context, is that also among premenopausal women breast cancer risk is directly related to serum testosterone concentrations, although among premenopausal women serum androgen levels are not a primary determinant of breast and tissue serum concentrations of estradiol (43;44), and no associations could be established so far, between premenopausal serum estrogen concentrations and breast cancer risk.

In summary, while breast cancer is a heterogeneous disease, we observed the widely reported associations of estradiol and its androgenic precursors with overall postmenopausal breast cancer risk were not statistically

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

heterogeneous between receptor positive tumors and hormone receptor negative breast cancer. Further research is needed to establish through which molecular pathways, and during which evolutionary stages of development, androgens and estrogens can promote the occurrence of both receptor-positive and –negative clinical breast tumors.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Funding

This study was (partly) funded by a grant from the German Research Foundation, Graduiertenkolleg 793: Epidemiology of communicable and chronic non-communicable diseases and their interrelationships. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue contre le Cancer, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Stavros Niarchos Foundation, the Hellenic Health Foundation and the Hellenic Ministry of Health. (Greece) ; Italian Association for Research on Cancer (AIRC) and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health. (Norway); Health Research Fund (FIS), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council, Stroke Association, British Heart Foundation, Department of Health, and Wellcome Trust (United Kingdom).

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Corresponding Author

Rudolf Kaaks, Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. Tel: +49 6221 422219; Fax: +49 6221 422203; Email: r.kaaks@dkfz.de

Conflict of interest declared

None

Acknowledgements

We would like to thank Miss Britta Lederer and Miss Sigrid Henke for their work in performing the immunoassays; Sabine Rohrmann, Jutta Schmidt and Jutta Kneisel for their assistance during the collection of hormone receptor status data; and all the EPIC cohort participants. The excellent comments from the two anonymous reviewers are also greatly acknowledged.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Reference List

(1) Weigelt B, Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? Nat Rev Clin Oncol 2009 Dec;6(12):718-30.

(2) Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 2004 Oct;13(10):1558-68.

(3) Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, Nicholson RI, Lee AH, Robertson JF, Ellis IO. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. J Clin Oncol 2007 Oct 20;25(30):4772-8.

(4) Geyer FC, Reis-Filho JS. Microarray-base gene expression profiling as a clinical tool for breast cancer management: Are we there yet? International Journal of Surgical Pathology 2008 Dec 22;17(4):285-302.

(5) Perou CM, Sorlie T, Eisen MB, van de RM, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, et al. Molecular portraits of human breast tumours. Nature 2000 Aug 17;406(6797):747-52.

(6) Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand S, Haibe-Kains B, Desmedt C, Ignatiadis M, Sengstag T, Schutz F, Goldstein DR, Piccart M, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res 2008;10(4):R65.

(7) Rosenberg LU, Einarsdottir K, Friman EI, Wedren S, Dickman PW, Hall P, Magnusson C. Risk factors for hormone receptor-defined breast cancer in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2006 Dec;15(12):2482-8.

(8) Chu KC, Anderson WF, Fritz A, Ries LA, Brawley OW. Frequency distributions of breast cancer characteristics classified by estrogen receptor and progesterone receptor status for eight racial/ethnic groups. Cancer 2001 Jul 1;92(1):37-45.

(9) Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002 Apr 17;94(8):606-16.

(10) Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, Dossus L, Lukanova A, Bingham S, Khaw KT, Allen NE, Bueno-de-Mesquita HB, et al. Postmenopausal serum androgens, oestrogens and breast cancer risk:

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

the European prospective investigation into cancer and nutrition. Endocr Relat Cancer 2005 Dec;12(4):1071-82.

(11) Zeleniuch-Jacquotte A, Toniolo P, Levitz M, Shore RE, Koenig KL, Banerjee S, Strax P, Pasternack BS. Endogenous estrogens and risk of breast cancer by estrogen receptor status: a prospective study in postmenopausal women. Cancer Epidemiol Biomarkers Prev 1995 Dec;4(8):857-60.

(12) Cummings SR, Lee JS, Lui LY, Stone K, Ljung BM, Cauleys JA. Sex hormones, risk factors, and risk of estrogen receptor-positive breast cancer in older women: a long-term prospective study. Cancer Epidemiol Biomarkers Prev 2005 May;14(5):1047-51.

(13) Baglietto L, Severi G, English DR, Krishnan K, Hopper JL, McLean C, Morris HA, Tilley WD, Giles GG. Circulating steroid hormone levels and risk of breast cancer for postmenopausal women. Cancer Epidemiol Biomarkers Prev 2010 Feb;19(2):492-502.

(14) Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. J Natl Cancer Inst 2004 Dec 15;96(24):1856-65.

(15) Sieri S, Krogh V, Bolelli G, Abagnato CA, Grioni S, Pala V, Evangelista A, Allemani C, Micheli A, Tagliabue G, Schunemann HJ, Menard S, et al. Sex hormone levels, breast cancer risk, and cancer receptor status in postmenopausal women: the ORDET cohort. Cancer Epidemiol Biomarkers Prev 2009 Jan;18(1):169-76.

(16) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 Suppl 1:S6-14.

(17) Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002 Dec;5(6B):1113-24.

(18) Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999 May;17(5):1474-81.

(19) McCann J. Better assays needed for hormone receptor status, experts say. J Natl Cancer Inst 2001 Apr 18;93(8):579-80.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

(20) Layfield LJ, Gupta D, Mooney EE. Assessment of Tissue Estrogen and Progesterone Receptor Levels: A Survey of Current Practice, Techniques, and Quantitation Methods. Breast J 2000 May;6(3):189-96.

(21) Flowers JL, Burton GV, Cox EB, McCarty KS, Sr., Dent GA, Geisinger KR, McCarty KS, Jr. Use of monoclonal antiestrogen receptor antibody to evaluate estrogen receptor content in fine needle aspiration breast biopsies. Ann Surg 1986 Mar;203(3):250-4.

(22) Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Pathologe 1987 May;8(3):138-40.

(23) Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999 Oct;84(10):3666-72.

(24) Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Riboli E, Toniolo P, Kaaks R. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev 2002 Oct;11(10 Pt 1):1065-71.

(25) Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003 Jun;6(4):407-13.

(26) Huedo-Medina TB, Sanchez-Meca J, Marin-Martinez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index? Psychol Methods 2006 Jun;11(2):193-206.

(27) Kahan Z, Gardi J, Nyari T, Foldesi I, Hajnal-Papp R, Ormandi K, Lazar G, Thurzo L, Schally AV. Elevated levels of circulating insulin-like growth factor-I, IGF-binding globulin-3 and testosterone predict hormone-dependent breast cancer in postmenopausal women: a case-control study. Int J Oncol 2006 Jul;29(1):193-200.

(28) von WR, Mengel M, Wiese B, Rudiger T, Muller-Hermelink HK, Kreipe H. Tissue array technology for testing interlaboratory and interobserver reproducibility of immunohistochemical estrogen receptor analysis in a large multicenter trial. Am J Clin Pathol 2002 Nov;118(5):675-82.

(29) Speroff L. Postmenopausal hormone therapy and breast cancer: a clinician's message for patients. Endocrine 2004 Aug;24(3):211-6.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

(30) Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab 2010 Jul;95(7 Suppl 1):s1-s66.

(31) Rinaldi S, Key TJ, Peeters PH, Lahmann PH, Lukanova A, Dossus L, Biessy C, Vineis P, Sacerdote C, Berrino F, Panico S, Tumino R, et al. Anthropometric measures, endogenous sex steroids and breast cancer risk in postmenopausal women: a study within the EPIC cohort. Int J Cancer 2006 Jun 1;118(11):2832-9.

(32) Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol 2001 Mar;2(3):133-40.

(33) Hankinson SE. Endogenous hormones and risk of breast cancer in postmenopausal women. Breast Dis 2005;24:3-15.

(34) Osborne CK, Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 2005 Mar 10;23(8):1616-22.

(35) Dickson RB, Stancel GM. Estrogen receptor-mediated processes in normal and cancer cells. J Natl Cancer Inst Monogr 2000;(27):135-45.

(36) Duffy MJ, O'Donovan N, Crown J. Use of molecular markers for predicting therapy response in cancer patients. Cancer Treat Rev 2011 Apr;37(2):151-9.

(37) Allred DC, Brown P, Medina D. The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer. Breast Cancer Res 2004;6(6):240-5.

(38) Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX, Pelletier G. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev 2003 Apr;24(2):152-82.

(39) Suzuki T, Miki Y, Nakamura Y, Moriya T, Ito K, Ohuchi N, Sasano H. Sex steroid-producing enzymes in human breast cancer. Endocr Relat Cancer 2005 Dec;12(4):701-20.

(40) Suzuki T, Miki Y, Takagi K, Hirakawa H, Moriya T, Ohuchi N, Sasano H. Androgens in human breast carcinoma. Med Mol Morphol 2010 Jun;43(2):75-81.

(41) Micello D, Marando A, Sahnane N, Riva C, Capella C, Sessa F. Androgen receptor is frequently expressed in HER2-positive, ER/PR-negative breast cancers. Virchows Arch 2010 Oct;457(4):467-76.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

(42) Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C, Gerald WL. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene 2006 Jun 29;25(28):3994-4008.

(43) Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, Secreto G, Amiano P, Bingham S, Boeing H, Bueno de Mesquita HB, Chang-Claude J, et al. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2005 May 18;97(10):755-65.

(44) Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, Hankinson SE. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 2006 Oct 4;98(19):1406-15.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

04.05.2011~~03.05.2011~~

Table 1: Baseline characteristics of case and control subjects, all centres combined, the EPIC cohort, 2007.

Covariate	Cases	Controls	P_{diff}*
Numbers of subjects	554	821	
ER-positive	382	602	
ER-negative	172	219	
PR-positive	211	345	
PR-negative	197	285	
ER+PR+	200	328	
ER+PR-	80	133	
ER-PR-	117	152	
Age at blood donation	59.8	60.1	
Years since menopause (blood donation)	10.7	11.1	
Years since menopause (diagnosis) - study phase 1	14.4	-	
Years since menopause (diagnosis) - study phase 2	15.9	-	
Years between blood donation and diagnosis	4.3	-	
Age at menarche	13.3	13.3	0.86
Age at menopause	49.3	49.0	0.50
Body-mass index (kg/m^2)	26.4	26	0.02
Waist circumference (cm)	83.1	82.5	0.11

*P for difference from paired t-test

Table 2: Geometric mean and 5th and 95th percentile ranges of case and control subjects, the EPIC cohort, 2007.

Hormones	Cases	Controls	P_{diff}*
Study phase 1	n = 329	n = 596	
Estradiol (pmol/L)	97.26(50.39-209.74)	88.63(43.78-189.41)	0.001
Free estradiol (pmol/L)	2.53(1.26-6.13)	2.27(1.01-5.01)	0.001
Testosterone (nmol/L)	1.25(0.52-2.85)	1.08(0.38-2.64)	0.0002
Free testosterone (pmol/L)	20.31(7.92-57.26)	17.12(4.60-49.02)	0.0002
SHBG (nmol/L)	34.26(15.41-77.23)	35.15(12.56-86.91)	0.46
Study phase 2	n = 225	n = 225	
Estradiol (pmol/L)	46.02(26.31-140.27)	40.66(25.91-79.27)	0.003
Free estrodiol (pmol/L)	1.08(0.56-3.62)	0.93(0.52-1.92)	0.001
Testosterone (nmol/L)	1.39(0.56-3.16)	1.18(0.52-2.60)	0.0001
Free testosterone (pmol/L)	18.89(7.11-51.23)	14.80(5.90-37.64)	<0.0001
SHBG (nmol/L)	45.67(18.68-105.0)	51.69(22.99-112.8)	0.01

*P for difference from paired t-test

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Table 3: Characteristic of nested matched case control studies on postmenopausal hormone concentrations and breast cancer risk by receptor status

Study	Cohort	No. of cases/ controls	ER+/ER-	PR+/PR-	ER+PR+/ER+PR-/ER-PR-	Hormones	Scale	Risk estimate**						
								ER-positive	ER-negative	PR-positive	PR-negative	ER+PR+	ER+/PR-	ER-PR-
Zeleniuch-Jacquotte et al (11)	New York University Women's Health Study	130/248	53/23	-	-	E2	Log_e continuous	1.9(0.9-4.0)	2.0(0.7-5.6)	-	-	-	-	-
						Free E2		3.3(0.6-20.3)	9.2(0.4-200.6)	-	-	-	-	-
						SHBG		1.0(0.7-1.4)	0.9(0.9-1.0)	-	-	-	-	-
Missmer et al (14)	Nurses Health Study	322/643	-	-	153/34/39	E2	Quartiles**	-	-	-	-	3.3(2.0-5.4)#	1.0(0.4-2.6)	1.0(0.4-2.4
						T		-	-	-	-	2.0(1.2-3.4)#	1.9(0.7-5.0)	0.7(0.3-1.6
						SHBG		-	-	-	-	0.5(0.3-0.8)#	1.8(0.6-5.0)	1.1(0.5-2.8
Cummings et al (12)	Osteoporotic Fractures Cohort *	208/378	196/-	-	-	E2	Quintiles**	2.9(1.6-5.1)	-	-	-	-	-	-
						T		5.1(2.5-10.3)	-	-	-	-	-	-
Kahán et al (27)	Hungarian Mammography Screening Program	102/102	-	-	64/23/10	T	Quartiles**	-	-	-	-	5.8(2.1-16.2)#	-	-
Sieri et al (15)	The ORDET Cohort	165/672	127/30	96/59	89/36/23	E2	Quartiles**	1.3(0.8-2.0)	0.9(0.4-2.2)	1.5(0.8-2.5)	0.9(0.5-1.7)	1.5(0.9-2.7)	0.8(0.4-1.8)	1.1(0.4-2.8
						T		2.7(1.6-4.3)#	1.6(0.7-3.8)	2.3(1.3-4.1)#	2.5(1.3-4.8)#	2.3(1.3-1.5)#	3.1(1.3-7.2)#	1.8(0.7-4.8
						Free T		1.6(1.0-2.6)#	1.9(0.7-4.8)	1.7(1.0-2.9)#	1.6(0.8-3.0)	1.8(1.0-3.1)#	1.2(0.5-2.6)	2.6(0.8-8.4
						SHBG		1.0(0.6-1.6)	0.8(0.3-2.0)	1.0(0.6-1.7)	0.8(0.4-1.6)	1.0(0.6-1.7)	1.0(0.4-2.3)	0.7(0.3-1.8
Baglietto et al (13)	Melbourne Collaborative Cohort *	197/857	132/45	87/89	83/48/41	E2	Log_2 continuous	1.5(0.9-2.5)	1.7(0.7-4.0)	1.5(0.8-2.8)	1.5(0.8-2.9)	1.7(0.9-3.1)	1.2(0.5-2.8)	2.0(0.8-5.0
						Free E2		1.7(1.1-2.6)	1.7(0.9-3.3)	2.0(1.2-3.3)	1.5(0.9-2.4)	2.3(1.4-3.8)	1.1(0.6-2.1)	2.1(1.1-4.2
						T		1.1(0.9-1.5)	1.0(0.7-1.5)	1.1(0.8-1.5)	1.1(0.8-1.5)	1.1(0.8-1.6)	1.2(0.8-1.7)	1.1(0.7-1.8
						SHBG		0.4(0.3-0.6)	0.4(0.2-0.8)	0.3(0.2-0.6)	0.5(0.3-0.8)	0.3(0.2-0.5)	0.6(0.4-1.1)	0.4(0.2-0.7

*Case cohort study design
**Estimates in categories are highest vs. lowest
#pvalue <0.05 on continuous trend
E2: Estradiol
Free E2: Free estradiol
T: Testosterone
Free T: Free testosterone

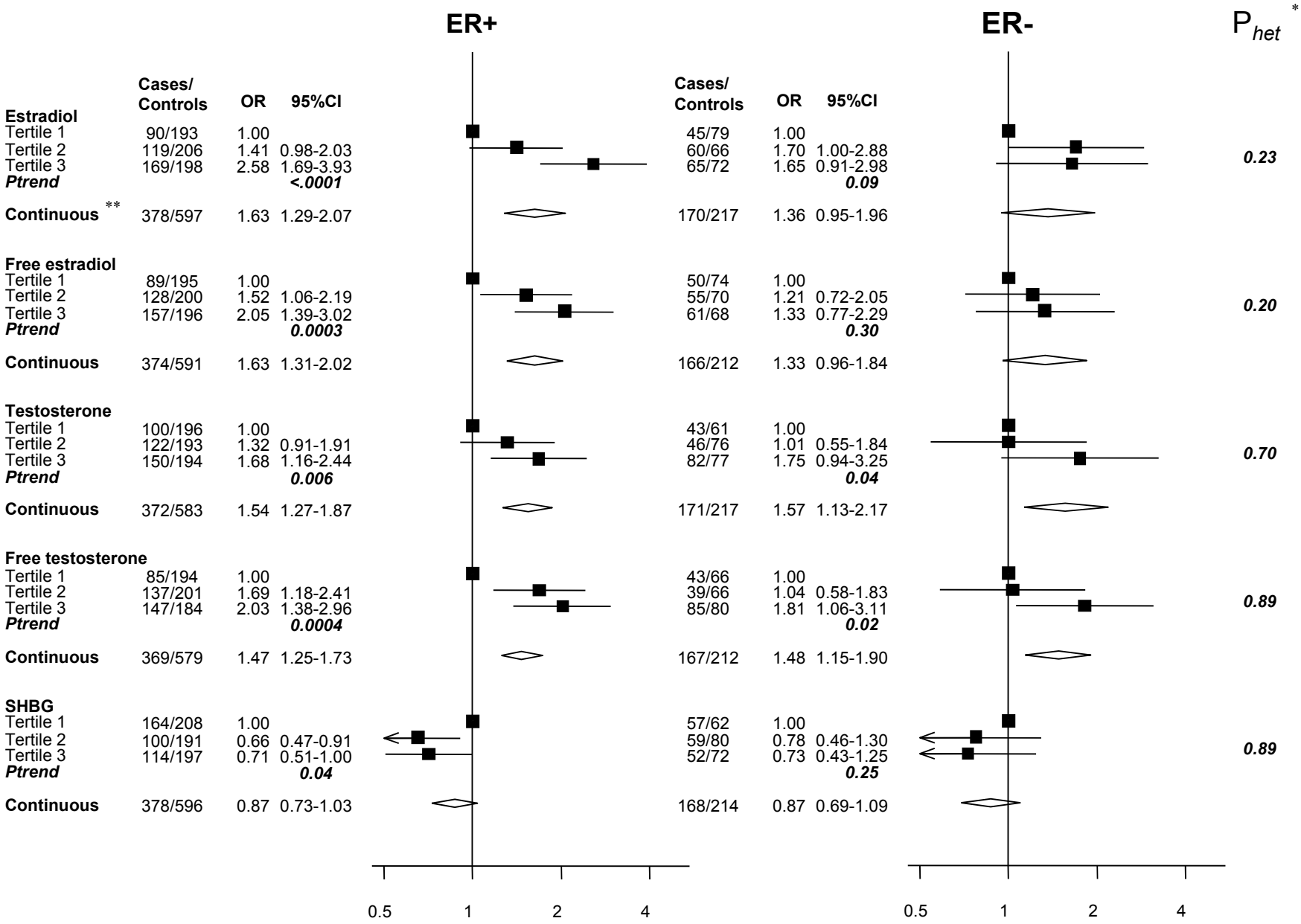

Figure 1: Odds ratio estimates for ER breast cancer subtypes in postmenopausal women according to tertiles of sex steroid hormone and SHBG levels.

Cases and controls matched for study center, batch, age at blood donation, time of blood collection and fasting status.

*Test for heterogeneity between ER-positive and ER-negative breast cancer was assessed using a log likelihood ratio test with and without an interaction term for breast cancer subtype outcome.

**Log2 continuous increase in circulating hormone level

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

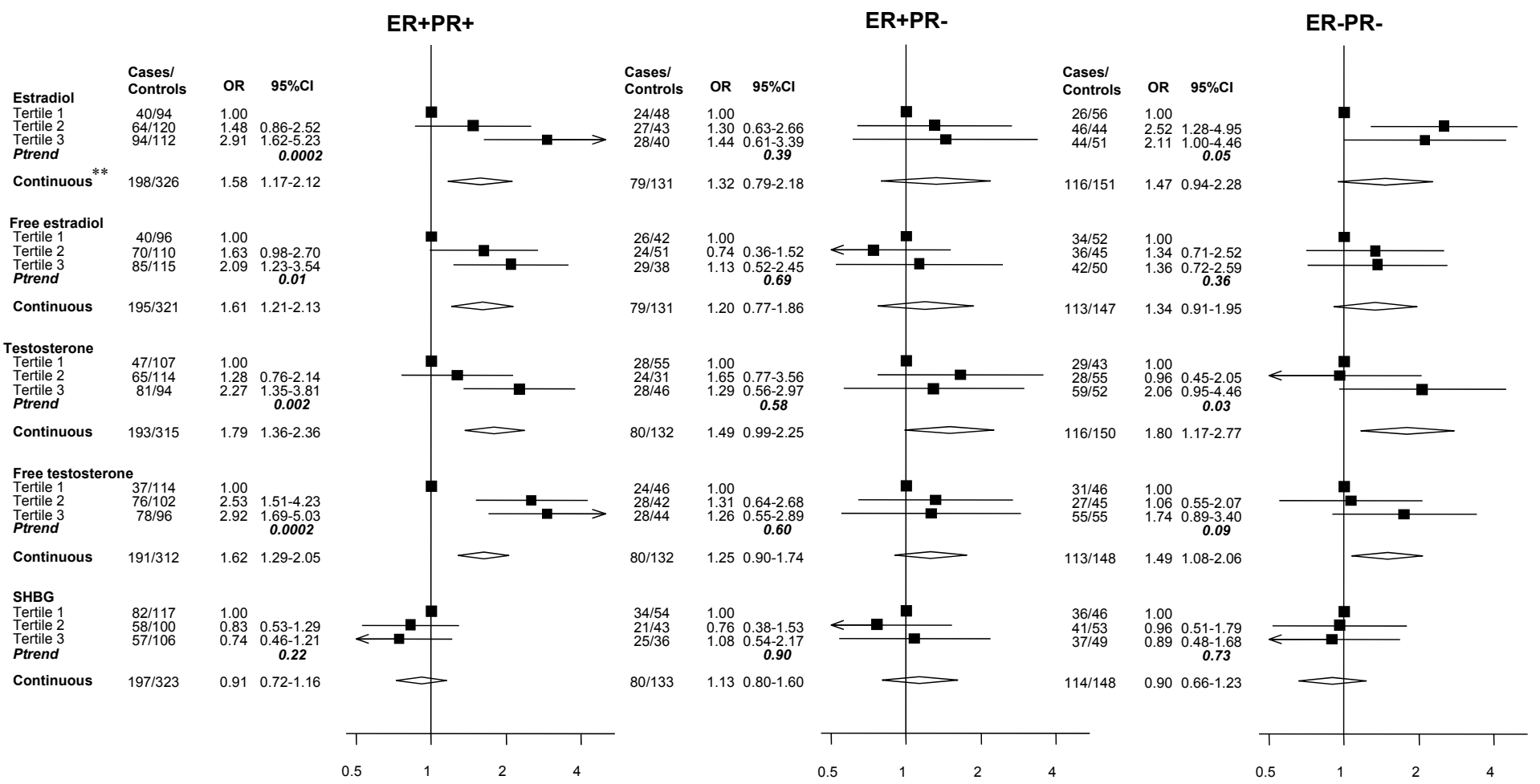

Figure 2: Odds ratio estimates for joint ERPR breast cancer subtypes in postmenopausal women according to tertiles of sex steroid hormone and SHBG levels.

Cases and controls matched for study center, batch, age at blood donation, time of blood collection and fasting status.

*Test for heterogeneity between ER+PR+, ER+PR- and ER-PR- breast cancer was assessed using a log likelihood ratio test with and without an interaction term for breast cancer subtype outcome.

**Log2 continuous increase in circulating hormone level

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Author Manuscript Published OnlineFirst on August 2, 2011; DOI: 10.1158/1940-6207.CAPR-11-0090
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

AACR American Association for Cancer Research

Cancer Prevention Research

Postmenopausal serum sex steroids and risk of hormone receptor positive and negative breast cancer: a nested case-control study

Rebecca E Ritte, Annekatrin Lukanova, Laure Dossus, et al.

Cancer Prev Res Published OnlineFirst August 2, 2011.

Updated version	Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-11-0090
Supplementary Material	Access the most recent supplemental material at: http://cancerpreventionresearch.aacrjournals.org/content/suppl/2011/08/02/1940-6207.CAPR-11-0090.DC1
Author Manuscript	Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/early/2011/07/29/1940-6207.CAPR-11-0090. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerpreventionresearch.aacrjournals.org on May 22, 2018. © 2011 American Association for Cancer Research.