Sex hormones and breast cancer risk in premenopausal women: collaborative reanalysis of seven prospective studies

Endogenous Hormones and Breast Cancer Collaborative Group

Running title: sex hormones and premenopausal breast cancer

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Abstract

Background

The relationships of circulating concentrations of oestrogens, progesterone and androgens with breast cancer and related risk factors in premenopausal women are not well understood.

Methods

Individual data on prediagnostic sex hormone and sex hormone binding globulin (SHBG) concentrations were contributed by 7 prospective studies. Analyses were restricted to women who were premenopausal and under age 50 at blood collection, and to breast cancer cases diagnosed before age 50. The odds ratios (ORs) with 95% confidence intervals (95% CIs) for breast cancer associated with hormone concentrations were estimated by conditional logistic regression in up to 767 cases and 1699 controls matched for age, date of blood collection, and day of cycle, with stratification by study and further adjustment for cycle phase. The associations of hormones with risk factors for breast cancer in control women were examined by comparing geometric mean hormone concentrations in categories of these risk factors, adjusted for study, age, phase of menstrual cycle and body mass index (BMI). All statistical tests were two-sided.

Findings

ORs for breast cancer associated with a doubling in hormone concentration were 1.19 (95% CI 1.06-1.35) for oestradiol, 1.17 (1.03-1.33) for calculated free oestradiol, 1.27 (1.05-1.54) for oestrone, 1.30 (1.10-1.55) for androstenedione, 1.17 (1.04-1.32) for DHEAS, 1.18 (1.03-1.35) for testosterone and 1.08 (0.97-1.21) for calculated free testosterone. Breast cancer risk was not associated with luteal phase progesterone (for a doubling in concentration OR=1.00 (0.92-1.09)), and adjustment for other factors had little effect on any of these ORs. The cross-sectional analyses in control women showed several associations of sex hormones with breast cancer risk factors.

Interpretation

Circulating oestrogens and androgens are positively associated with the risk for breast cancer in premenopausal women, and may mediate some of the effects of other risk factors on breast cancer.

Keywords: Breast cancer; sex hormones; premenopausal

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INTRODUCTION

Breast cancer risk is affected by several reproductive and hormonal factors and it has long been hypothesized that endogenous sex hormones influence risk.[1] There are now sufficient data from studies of hormones and breast cancer risk in postmenopausal women to show that risk is positively associated with circulating concentrations of oestrogens and androgens,[2–4] but in premenopausal women fewer data are available and hormone measurements are complicated by the substantial variation in hormone concentrations across the menstrual cycle.

The Endogenous Hormones and Breast Cancer Collaborative Group was established to conduct pooled analyses of individual data from prospective studies in order to increase the precision of the estimated associations of endogenous hormones with breast cancer risk.[2] We report here a collaborative analysis of data from seven studies. We describe the associations of circulating sex hormones with breast cancer risk, including examination of consistency between studies, associations in subgroups, and the effects of adjustment for other risk factors. We also describe cross-sectional analyses of the associations of circulating sex hormones and sex hormone binding globulin (SHBG) with risk factors for breast cancer. The aim was to further understanding of the role of sex hormones in the aetiology of premenopausal breast cancer, therefore all analyses were restricted to women who were premenopausal and aged under 50 years at blood collection, and to case-control sets where the case was diagnosed with breast cancer before age 50.

METHODS

Data collection

Published studies were eligible for the collaborative re-analysis if they included data on endogenous hormones and breast cancer risk using prospectively collected blood samples from premenopausal women. Studies were identified by computer aided literature searches and through discussions with colleagues. The studies included were: CLUE I, Washington County, MD, USA;[5,6] Columbia, MO, USA;[7] European Prospective Investigation into Cancer and Nutrition (EPIC), Europe;[8] Guernsey, UK;[9] Nurses' Health Study II (NHS-II), USA;[10–12] New York University Women's Health Study (NYU WHS), USA;[13] Study of Hormones and Diet in the Etiology of Breast Tumors (ORDET), Italy.[14] The majority of the women in these studies were of white European ethnic origin. Two further studies in the Collaborative Group had prospective hormone data but were not included in the analyses reported here because data on day of menstrual cycle at blood collection were not available: Melbourne Collaborative Cohort Study (MCCS), Australia,[15] and Radiation Effects Research Foundation (RERF) study phases 1 and 2, Japan.[16,17] One further study was identified but the data could not be retrieved; this study included 17 cases of breast cancer and 67 matched controls among women who were premenopausal at blood collection.[18]

Table 1 summarizes the study designs. Details of the recruitment of participants, informed consent, ethics approvals and definitions of reproductive variables are in the

original publications. The majority of cases were of invasive breast cancer, but some studies also included *in situ* cases. Cases were individually matched to between 2 and 4 controls: all studies matched on age and date of blood sample (or follow-up time for EPIC), and on the day or phase of menstrual cycle at blood collection. Collaborators were asked to provide data on concentrations of the hormones oestradiol (total), oestrone, progesterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), testosterone, and SHBG, as well as data on reproductive, anthropometric and lifestyle factors for each woman in their study, where available. Women who were using hormonal contraceptives or other exogenous sex hormones at the time of blood collection were excluded, as were women missing information for date of birth, date at blood collection, day of menstrual cycle at blood collection, or date of diagnosis (cases).

Brief details of the assays are in Web Table 1, with further details in the original publications. Six studies measured hormone concentrations in serum whereas one (NHS II) used heparin plasma; for convenience we refer to serum concentrations for the pooled analyses. Circulating concentrations of free oestradiol and free testosterone were calculated from the concentrations of oestradiol and testosterone respectively and of SHBG, with albumin assumed to be constant (40 g/L), according to the law of mass action.[19,20]

Statistical analysis

Day of cycle at blood collection was grouped into six categories, using days until next period if available (backward dating), otherwise using days since last period (forward dating). The six categories were: early follicular (days 24+ backwards, or days 1-5 forwards), late follicular (19-23 backwards, 6-10 forwards), mid-cycle (15-18 backwards, 11-14 forwards), early luteal (11-14 backwards, 15-18 forwards), mid luteal (5-10 backwards, 19-24 forwards), late luteal (0-4 backwards, 25+ forwards). For CLUE I and Columbia, day of cycle was determined using forward dating for all participants. For Guernsey and NHS II, day of cycle was determined backwards for all participants (except one case in NHS II). For the other three studies, the percentages determined using backward dating (otherwise forward dating) were: 54.4% and 51.0% in cases and controls respectively in EPIC; 75.2% and 82.9% in cases and controls in NYU WHS; and 94.0% and 96.1% in cases and controls in ORDET.

In NHS-II participants provided two blood samples at baseline, one collected in the follicular phase and one in the luteal phase. For most of the analyses reported here we use values for oestradiol and oestrone from the follicular phase, and progesterone, androstenedione, DHEAS, testosterone and SHBG from the luteal phase; in the analyses of oestradiol subdivided by phase of cycle (Figure 1 and Web Figure 12) we used both the follicular and the luteal measures.

All women were classified as premenopausal in the contributed datasets, with the criteria for this based on questionnaire information, as described in the original studies; four studies additionally measured serum FSH concentration and excluded women with FSH values higher than the cut-off recommended by their laboratory (Guernsey, NYU WHS, ORDET, and Columbia for women aged 45 and above with

missing date of last menses). We restricted the analyses to cases diagnosed before age 50 (and their matched controls), so that most cases would have been diagnosed when premenopausal; this restriction further served to reduce the possibility that some participants were perimenopausal at blood collection.

All the studies used a nested case-control design, with assays arranged so that case-control sets were generally measured in the same batch, thus eliminating inter-assay variation from the case-control comparisons. We retained the original matched sets in the analyses. Some studies used density sampling, meaning that an individual participant could appear more than once in a data file.

Conditional logistic regression was used to calculate the odds ratio (OR) for breast cancer in relation to the serum concentrations of hormones and SHBG, categorizing women in each study according to the quintiles of hormone concentration for the controls in that study, after standardizing for phase of menstrual cycle using residuals from the study-specific mean for each cycle phase; for progesterone we restricted the analysis to samples collected in the luteal phase. Study-specific quintile cut-points were used because the absolute concentrations of hormones and SHBG vary between studies due to laboratory variation; further explanation of this approach is provided in previous publications.[2,21] To test for the significance of the association and to provide a summary measure of risk we also calculated the odds ratio associated with a unit increase in a continuous variable equal to the logarithm to the base 2 of the hormone concentration. A unit increase in this variable is equivalent to a doubling in hormone concentration. Heterogeneity in linear trends between studies was tested by comparing the chi-squared values for models with and without a (study) x (linear trend) interaction term. We also used chi-squared tests to examine whether there was evidence of heterogeneity in the associations of hormones with breast cancer risk according to subgroups defined by years between blood collection and diagnosis, stage of disease, receptor status and other characteristics. We also investigated the associations of hormones with breast cancer risk after adjusting for reproductive and hormonal risk factors for breast cancer: age at menarche (<12, 12-13, 14+ years, unknown); parity (zero, 1, 2, 3, 4+ full-term pregnancies, unknown); age at first full-term pregnancy (<20, 20-24, 25-29, 30+ years, unknown); body mass index (BMI; <22.5, 22.5-24.9, 25.0-27.4, 27.5-29.9, 30.0+ kg/m^2, unknown).

Concentrations of the hormones and SHBG were positively skewed, therefore log-transformed concentrations were used for all parametric analyses. Correlations between hormones were calculated using standardised log-transformed concentrations within each study, the standardised values being calculated by subtracting the mean log concentration and dividing by the standard deviation of the log concentration. The associations of hormones with risk factors for breast cancer were examined in the controls. Geometric means and 95% confidence intervals were calculated according to categories of these factors, adjusting for study, age (<40, 40-44, 45-49), cycle phase, and body mass index (BMI), as appropriate. F-tests were used to test for heterogeneity in the geometric mean hormone concentrations between the categories of risk factors, and where appropriate to test for trends across the categories, with the ordered categories scored from 1 to the maximum number of categories. The heterogeneity between studies in the associations of hormones with risk factors was assessed by adding a (study) x (factor) interaction term to the model and using the F-test to calculate its significance.

All statistical tests were two-sided and statistical significance was set at the 5% level. All analyses were performed using Stata version 12.0 (Stata Corp., College Station, TX).

Role of the funding source

The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The members of the writing team had full access to all data in the study. The corresponding author had the final responsibility for the decision to submit for publication.

RESULTS

Characteristics of cases and controls

Mean age at blood collection ranged from 35.6 years for cases in CLUE to 42.2 years for cases and controls in EPIC (Web Table 2). The median time between blood collection and diagnosis ranged from 2 years in EPIC to 9 years in CLUE. Geometric mean concentrations of sex hormones and SHBG in controls ranged from 164 to 316 pmol/L for oestradiol, 1.85 to 4.25 pmol/L for calculated free oestradiol, 145 to 360 pmol/L for oestrone, 9.62 to 43.5 nmol/L for luteal phase progesterone, 2.88 to 5.79 nmol/L for androstenedione, 2208 to 3921 nmol/L for DHEAS, 0.84 to 1.56 nmol/L for testosterone, 10.1 to 23.3 pmol/L for calculated free testosterone, and 43.0 to 74.3 nmol/L for SHBG (Table 2).

Sex hormones, SHBG and breast cancer risk

Figure 1 shows geometric mean concentrations of oestradiol and progesterone in cases and controls by phase of menstrual cycle at blood collection. For oestradiol, geometric mean values in cases were higher than in controls at all cycle phases except the late luteal phase. For progesterone, geometric means were lower in cases than controls in the early luteal phase, with small, non-significant differences in other phases.

Oestradiol, calculated free oestradiol, oestrone, androstenedione, DHEAS and testosterone were positively associated with breast cancer risk, with ORs in the top fifth of the distribution of 1.41, 1.19, 1.50, 1.68, 1.45, and 1.32, respectively (Figure 2); using a doubling scale, the ORs were 1.19 for oestradiol, 1.17 for calculated free oestradiol, 1.27 for oestrone, 1.30 for androstenedione, 1.17 for DHEAS, and 1.18 for testosterone. Luteal phase progesterone, calculated free testosterone and SHBG were not significantly associated with risk. In a sensitivity analysis restricted to women with blood collected at ages below 45 and cases diagnosed before age 45 the results were similar (Web Figure 1). There was no significant heterogeneity between studies in the associations of these hormones with breast cancer risk (Web Figures 2 to 10). Further adjustment for age at menarche, age at first full-term pregnancy, number of full-term pregnancies, and BMI did not substantially change the ORs, except that after

adjustment there was a statistically significant positive association of calculated free testosterone with risk (OR 1.14, P = 0.031; Web Figure 11).

Subgroup analyses

Subgroup analyses were conducted to detect heterogeneity in the associations of log hormone concentrations with breast cancer risk in subgroups according to years from blood collection to diagnosis (< 4, ≥4), stage of disease (*in situ*, invasive), oestrogen receptor status (positive, negative), progesterone receptor status (positive, negative), HER2 receptor status (positive, negative), phase of menstrual cycle at blood collection (except for progesterone; follicular, mid-cycle, luteal), age at menarche (<14, ≥14 years), parity (nulliparous, parous), age at first full-term pregnancy (<25, ≥25 years), mother or sister with breast cancer (no, yes), BMI (<25, ≥25 kg/m²), smoking (never or past, current), alcohol intake (<10, ≥10 g/d), previous use of hormonal contraceptives (no, yes), and assay method for oestradiol, calculated free oestradiol, oestrone, testosterone and calculated free testosterone (extraction, non-extraction); see Web Figures 12 to 20. Of the nine hormones examined in relation to these factors, four out of 130 tests for heterogeneity were statistically significant: for oestradiol the OR for a doubling in concentration was 1.26 (1.10-1.44) for never or past smokers and 0.94 (0.75-1.18) for current smokers (P for heterogeneity=0.034), and 1.01 (0.82-1.24) for never users and 1.32 (1.14-1.53) for past users of hormonal contraceptives (P for heterogeneity=0.030); for oestrone the OR for a doubling in concentration was 1.74 (0.99-3.03) for progesterone receptor positive and 0.54 (0.27-1.08) for progesterone receptor negative cancers (P for heterogeneity=0.010); and for luteal phase progesterone the OR for a doubling in concentration was 1.25 (1.01-1.55) for nulliparous women and 0.99 (0.90-1.09) for parous women (P for heterogeneity=0.034).

Two sub-group analyses were of particular *a priori* interest. For oestrogens and androgens, the ORs were larger for oestrogen receptor positive tumours than for oestrogen receptor negative tumours, but none of these differences was statistically significant (Table 3). For oestradiol according to phase of menstrual cycle the ORs for a doubling in concentration were 1.25 (1.06-1.48) for follicular, 1.20 (0.81-1.79) for mid-cycle, and 1.13 (0.92-1.37) for luteal samples, P for heterogeneity=0.732 (Web Figure 12).

Associations of hormones and SHBG with BMI, parity and other factors

BMI was inversely associated with oestradiol, luteal phase progesterone and SHBG, with mean concentrations 17%, 28%, and 46% lower, respectively, in women with a BMI of 30 and above compared to women with a BMI of under 22.5 kg/m²; conversely, calculated free oestradiol, oestrone, DHEAS, testosterone and calculated free testosterone were positively associated with BMI, with mean concentrations 10%, 16%, 8%, 7% and 63% higher, respectively, in women with a BMI of 30 and above compared to women with a BMI of under 22.5 kg/m² (means adjusted for age, study and cycle phase; Figure 3).

The associations of sex hormones with age, age at menarche, parity, family history of breast cancer, smoking, alcohol and previous use of hormonal contraceptives are

shown in Web Figures 21 to 27. Sex hormone concentrations were lower in older than in younger women, whereas SHBG was higher in older women (Web Figure 21). Parity was inversely associated with calculated free testosterone, but was not significantly associated with concentrations of the other sex hormones or SHBG (Web Figure 23), and none of the hormones or SHBG was significantly associated with age at menarche or family history of breast cancer (Web Figures 22 and 24). Androstenedione, DHEAS, testosterone and calculated free testosterone were higher in current smokers of 15+ cigarettes per day than in never-smokers, by 21%, 12%, 12% and 13% respectively (Web Figure 25), and the same hormones were positively associated with alcohol consumption, with mean concentrations 14%, 16%, 23% and 23% higher, respectively, in women with an alcohol intake of 20 g/d and above compared to women who did not consume alcohol (Web Figure 26); further adjustment of the analyses by smoking for alcohol, and of the analyses by alcohol for smoking, had no material effect on the results (not shown). Women who had previously used hormonal contraceptives had lower concentrations of oestradiol (by 7%), oestrone (by 7%), androstenedione (by 5%) and SHBG (by 4%) (Web Figure 27).

DISCUSSION

Sex hormones, SHBG and breast cancer risk

Oestradiol, calculated free oestradiol, oestrone, androstenedione, DHEAS and testosterone were positively associated with breast cancer risk, whereas luteal phase progesterone and SHBG were not associated with risk. Calculated free testosterone was positively associated with breast cancer risk in the adjusted analysis, but not in the unadjusted analysis. These associations did not vary according to the time between blood collection and diagnosis, making reverse causality unlikely, and (with the exception of calculated free testosterone) were not materially affected by adjustment for other risk factors, suggesting that confounding is unlikely. These results therefore strongly suggest that breast cancer risk in premenopausal women increases with increasing concentrations of these sex hormones. The results are qualitatively similar to those reported in postmenopausal women, but smaller in magnitude.[2–4]

The analyses reported in this paper were all based on a single hormone measure for each woman. Measurements of hormone concentrations are subject to largely random error associated with assay variation and fluctuations in serum levels within individual women. Studies of the reproducibility of sex hormones in premenopausal women for up to three years have shown intra-class correlations of ~0.6 or above for androgens and SHBG, but correlations of ~0.4 or less for oestrogens and progesterone.[22,23] It is therefore likely that the observed associations between hormone concentrations and breast cancer risk are underestimates of the true associations, particularly for oestrogens, but more reproducibility data are required.

The sub-group analyses showed heterogeneity in the associations of oestradiol with risk according to smoking and previous use of hormonal contraceptives, of oestrone with risk according to progesterone receptor status, and of luteal phase progesterone with risk according to parity, but there was no significant heterogeneity according to

any other combination of factor and hormone. All the sex hormones had larger associations with the risk of oestrogen receptor positive breast cancer than with the risk of oestrogen receptor negative disease; these differences were not significant, but statistical power was low due to small numbers of oestrogen receptor negative disease (e.g. 71 cases for oestradiol). For oestradiol the plot of geometric mean concentrations in cases and controls according to phase of menstrual cycle (Figure 1) suggested that concentrations in cases were higher than those in controls in the follicular phase and at mid-cycle, but not in the late luteal phase, and similarly the sub-group analyses of breast cancer risk showed larger ORs in the follicular phase and at mid-cycle than in the luteal phase, but these differences were not significant.

Associations of hormones with breast cancer risk factors in controls

All the hormones, except for androstenedione, were associated with BMI. Total oestradiol was inversely associated with BMI, whereas free oestradiol was positively associated with BMI because of the strong inverse association of SHBG with BMI. Interpretation of these observations is difficult, but if free oestradiol is a reliable index of bioavailable oestradiol then obese premenopausal women are exposed to a slightly more oestrogenic environment. Oestrone was also positively associated with BMI, perhaps due to increased peripheral aromatization of androstenedione, as in postmenopausal women.[24] Progesterone was lower in obese than non-obese women, whereas DHEAS and testosterone were positively associated with BMI. Similar findings for oestrogens and progesterone have been reported among regularly menstruating women in the BioCycle Study,[25] and in massively obese premenopausal women.[26]

Parity was not strongly associated with any of the hormones, but showed an inverse association with calculated free testosterone. Some previous studies in younger premenopausal women have suggested that early menarche and nulliparity are associated with oestrogen levels,[27,28] but in the current study none of the hormones or SHBG was significantly associated with age at menarche, and none of the oestrogen measures was associated with parity.

Androstenedione, DHEAS, testosterone and free testosterone were higher in women who consumed the most cigarettes and the most alcohol than in non-smokers and non-drinkers, respectively. Very similar associations were seen in postmenopausal women.[29] The mechanism may involve stimulation of hormone synthesis by the adrenal glands.[30]

Women who had previously used hormonal contraceptives had lower concentrations of oestradiol, oestrone, androstenedione, and SHBG. It is not clear whether these are causal associations, or what mechanism could be involved, though they might involve long-term effects on the liver.[31]

Sex hormones may mediate the effects of some risk factors on the development of breast cancer. For example, the increase in breast cancer risk caused by alcohol[32] might be due to increased serum concentrations of sex hormones, although it could also be due to other effects of alcohol. BMI is inversely associated with the risk of breast cancer in premenopausal women,[33] and this might be related to the effects of

obesity on hormone levels. We observed that total oestradiol was inversely related to BMI and positively associated with risk, which is compatible with the idea that the lower risk in obese women is due to lower oestradiol, but this interpretation is complicated by the fact that we observed that free oestradiol was positively associated with BMI, as were oestrone and the androgens DHEAS, testosterone and free testosterone. Luteal phase progesterone was also lower in obese than normal weight women, perhaps due to a higher probability of anovulatory cycles in obese women;[34] our analyses do not show any association of progesterone with breast cancer risk, but the reliability of progesterone measurements is low and more data are needed before concluding that progesterone is not a determinant of breast cancer risk.

The strengths of this analysis are that the data and serum samples were all collected prospectively, that it includes almost all the available data from published studies world-wide, and that we were able to adjust for phase of cycle and for other potential risk factors. The total sample size is moderately large for most of the hormones, but power is low for the sub-group analyses.

A potential weakness is that the study designs and methods for measuring hormones and other risk factors were not standardized. For example, studies variably used forward or backward dating in determining when blood was collected in the menstrual cycle, and, because of differences in progesterone measurement across study, we were unable to distinguish ovulatory versus anovulatory cycles. Further, hormone concentrations varied substantially between studies, and this is likely to reflect, in part, differences in assay methods. The accuracy of assay methods varies, and assays which incorporate an extraction step are more accurate than "direct" non-extraction assays.[35] Ideally assays would be standardized and use the most accurate methods available, but in the current analysis our aim was to make the best use of the data available. To allow for differences in absolute hormone concentrations between assay laboratories we used study-specific quintiles of hormone concentrations.[21] This approach assumes that the true concentrations across the quintiles are similar in all the studies, and if this assumption is not correct then the estimates of ORs may be biased. However, because heterogeneity in risk estimates was not evident between studies or between assay methods (extraction versus non-extraction) this assumption does seem reasonable.

This collaborative analysis shows a positive association between sex hormones and breast cancer risk in premenopausal women. It is not known whether this association is causal, but there are plausible biological mechanisms which could explain such an effect. The magnitude of the observed association is modest, but the true association may be substantially larger because of measurement error in the assessment of long-term premenopausal hormone levels. Further research is needed to provide more robust estimates of the overall associations and associations in sub-groups, and to determine the environmental and genetic factors that cause differences in hormone levels between premenopausal women.

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MEMBERS AND AFFILIATIONS OF THE ENDOGENOUS HORMONES AND BREAST CANCER COLLABORATIVE GROUP

All named members of the collaborative group are authors of this manuscript. TJ Key co-ordinated the collaborative group, drafted the manuscript, conducted the literature search and contributed to study design and data interpretation. PN Appleby centralized the data, conducted the statistical analyses, and contributed to interpreting the data. GK Reeves and RC Travis contributed to study design, data interpretation and writing. All the co-authors from the collaborating studies contributed to data collection, data interpretation and writing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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Legends to Figures

Fig. 1. Geometric mean oestradiol and progesterone concentrations (pmol/L and nmol/L, respectively, with 95% confidence intervals) in cases and controls by phase of menstrual cycle. Adjusted for study and age at blood collection. EF=early follicular; LF=late follicular; MC=mid cycle; EL=early luteal; ML=mid luteal; LL=late luteal.

Fig. 2. Odds ratios (ORs) for breast cancer associated with sex hormones and SHBG. The black squares indicate the ORs in fifths (study-specific fifths after adjustment for phase of cycle within each study), and the horizontal lines show the 95% confidence intervals. The area of each square is proportional to the amount of statistical information (inverse of the variance of the logarithm of the OR). The diamonds show the OR for a doubling in concentration, and the widths of the diamonds show the 95% confidence intervals. Estimates are from conditional logistic regression on case-control sets matched within each study.

Fig. 3. Geometric mean hormone and SHBG concentrations (with 95% confidence intervals) in controls by BMI. Adjusted for study, age at blood collection and phase of menstrual cycle.

Table 1. Description of studies

Study	Recruitment period	Fasting status	Storage temperature	Matching criteria				
				Controls per case	Age at blood collection	Date of blood sample	Day of cycle	Other and comments
CLUE I, USA	1974	Non-fasting	-70 C	2	± 1 year	± 14 days	± 1 day	Time of day, fasting status, ethnic group, freeze/thaw history of serum sample
Columbia, USA	1977-1989	Non-fasting	-70 C	2	± 2 years	± 1 year	± 2 days	Time of day at blood collection
EPIC, Europe	1992-1998	Matched	Mostly -196 C[1]	2	± 6 months	No (incidence density sampling)	5 phases	Time of day at blood collection, subcohort
Guernsey, UK	1977-1990	Non-fasting	-20 C	3	± 2 years	± 1 year	± 1 day	
Nurses' Health Study II phases 1 (1999-2003 follow-up cycles) and 2 (2005-2009 follow-up cycles), USA	1996-1999	Matched	-130 C	2	± 2 years	± 2 months	± 1 day for luteal blood sample (asked to provide follicular sample at days 3 to 5 and luteal sample at 7-9 days before anticipated start of next cycle)	Time of day, fasting status
NYU WHS, USA	1985-1991	Non-fasting	-80 C	2	± 6 months	± 3 months	5 phases and day	Number of subsequent samples
ORDET, Italy	1987-1992	12 hour fast prior to collection. Samples taken 07.30-09.00	-80 C	4	± 5 years	± 89 days	All days 20 to 24	Daylight saving period, recruitment centre

[1] Stored in liquid nitrogen at -196 C, except in Denmark in nitrogen vapour at -150 C.
CLUE I = Washington County, MD Study "Give us a clue to cancer and heart disease"; EPIC = European Prospective Investigation into Cancer and Nutrition; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors.

Table 2. Geometric mean hormone concentrations (95% CI) by study and case-control status

Study		Number[1]	Oestradiol, pmol/L	Calculated free oestradiol, pmol/L	Oestrone, pmol/L	Luteal phase progesterone, nmol/L	Androstenedione, nmol/L	DHEAS, nmol/L	Testosterone, nmol/L	Calculated free testosterone, pmol/L	SHBG, nmol/L
CLUE I, USA	Cases	21	172 (134-222)	2.03 (1.55-2.64)	252 (211-301)	5.32 (1.73-16.3)	2.98 (2.31-3.85)	3903 (2787-5465)	-	-	69.9 (58.0-84.2)
	Controls	42	168 (137-206)	1.85 (1.51-2.26)	239 (207-275)	9.62 (5.47-16.9)	2.88 (2.42-3.43)	3853 (3023-4910)	-	-	74.3 (65.3-84.4)
Columbia, USA	Cases	13	239 (165-347)	3.26 (2.24-4.75)	-	-	-	-	1.00 (0.79-1.28)	13.7 (9.86-19.1)	48.2 (34.3-67.7)
	Controls	24	316 (257-387)	4.05 (3.34-4.92)	-	-	-	-	0.86 (0.73-1.02)	10.7 (9.10-12.7)	56.6 (48.3-66.4)
EPIC, Europe	Cases	206	318 (285-355)	4.60 (4.13-5.12)	384 (354-416)	8.42 (6.30-11.3)	5.59 (5.22-5.98)	3712 (3469-3972)	1.70 (1.60-1.81)	25.2 (23.2-27.3)	43.5 (40.6-46.6)
	Controls	408	296 (275-318)	4.25 (3.94-4.60)	360 (339-383)	12.3 (9.84-15.4)	4.92 (4.68-5.18)	3341 (3169-3522)	1.56 (1.49-1.63)	23.3 (21.8-24.8)	43.0 (40.9-45.3)
Guernsey, UK	Cases	32	323 (253-412)	3.16 (2.39-4.17)	-	10.7 (5.84-19.5)	-	2253 (1410-3599)	1.17 (0.97-1.40)	13.2 (11.3-15.5)	68.6 (59.5-79.1)
	Controls	94	282 (246-323)	3.02 (2.52-3.62)	-	10.6 (7.25-15.4)	-	2548 (1924-3375)	1.12 (1.02-1.23)	13.4 (11.6-15.5)	61.5 (55.8-67.7)
Nurses' Health Study II phase 1, USA	Cases	139	182 (166-199)	2.30 (2.12-2.49)	150 (142-159)	45.7 (41.1-50.8)	3.91 (3.68-4.16)	2302 (2129-2489)	0.92 (0.87-0.99)	11.3 (10.5-12.2)	57.9 (53.8-62.3)
	Controls	268	164 (153-177)	2.08 (1.95-2.22)	145 (138-151)	43.5 (39.9-47.4)	3.89 (3.72-4.06)	2208 (2089-2333)	0.90 (0.86-0.94)	10.9 (10.3-11.5)	58.5 (55.5-61.8)
Nurses' Health Study II phase 2, USA	Cases	105	193 (175-213)	2.21 (2.02-2.42)	161 (150-173)	40.7 (34.6-47.9)	-	2838 (2556-3151)	0.91 (0.85-0.98)	9.6 (8.7-10.5)	70.6 (65.3-76.3)
	Controls	203	186 (174-199)	2.25 (2.11-2.40)	163 (154-171)	38.1 (33.9-42.9)	-	2642 (2449-2851)	0.91 (0.87-0.96)	10.6 (10.0-11.3)	62.4 (59.0-66.0)
NYU WHS phase2, USA	Cases	137	-	-	-	-	4.30 (3.96-4.67)	3978 (3625-4366)	1.01 (0.91-1.12)	14.0 (12.4-15.8)	48.1 (44.1-52.4)
	Controls	258	-	-	-	-	4.07 (3.83-4.33)	3869 (3598-4161)	0.95 (0.88-1.03)	13.1 (11.9-14.3)	47.8 (44.8-51.0)
ORDET, Italy	Cases	84	300 (274-329)	3.66 (3.34-4.00)	-	38.2 (32.7-44.6)	5.26 (4.38-6.32)	3856 (3153-4715)	0.85 (0.75-0.97)	9.9 (8.5-11.6)	62.0 (56.6-68.0)
	Controls	336	282 (259-306)	3.50 (3.23-3.79)	-	32.4 (28.3-37.1)	5.79 (5.38-6.23)	3921 (3604-4265)	0.84 (0.79-0.90)	10.1 (9.3-10.8)	59.8 (57.1-62.6)

[1]Numbers are for women with known phase of cycle and values for oestradiol (except for NYU WHS where numbers are for women with values for testosterone).
- indicates data not available.
Geometric mean hormone concentrations for Nurses' Health Study II are obtained using the follicular phase data for oestradiol, calculated free oestradiol and oestrone and using the luteal phase data for all other hormones.
CLUE I = Washington County, MD Study "Give us a clue to cancer and heart disease"; EPIC = European Prospective Investigation into Cancer and Nutrition; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors.

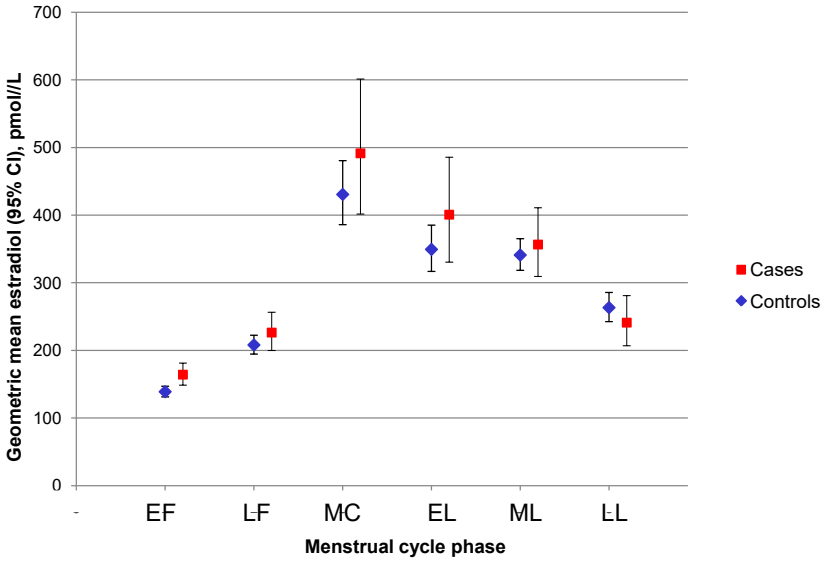
Table 3. Associations of hormones and SHBG with breast cancer risk, subdivided by oestrogen receptor status

	ER positive		ER negative		
Hormone	**Cases/controls**	**OR (95% CI)**	**Cases/controls**	**OR (95% CI)**	**P for heterogeneity**
Oestradiol	147/374	1.25 (0.95-1.65)	71/209	1.09 (0.76-1.57)	0.56
Calculated free oestradiol	147/374	1.22 (0.91-1.63)	71/209	1.03 (0.68-1.54)	0.50
Oestrone	107/205	1.26 (0.77-2.06)	37/72	0.90 (0.45-1.82)	0.45
Luteal phase progesterone	152/369	1.05 (0.88-1.24)	67/184	1.13 (0.88-1.47)	0.62
Androstenedione	124/237	1.45 (0.98-2.15)	54/106	1.11 (0.58-2.14)	0.50
DHEAS	170/327	1.24 (0.97-1.57)	67/130	0.91 (0.62-1.34)	0.19
Testosterone	211/495	1.13 (0.88-1.43)	99/265	1.03 (0.76-1.39)	0.66
Calculated free testosterone	211/495	1.08 (0.88-1.33)	99/264	1.01 (0.78-1.30)	0.66
SHBG	214/503	1.04 (0.80-1.35)	102/271	1.08 (0.77-1.52)	0.86

Odds ratios (ORs) for breast cancer associated with a doubling in concentration.
Estimates are from conditional logistic regression on case-control sets matched within each study.

Figure 1a

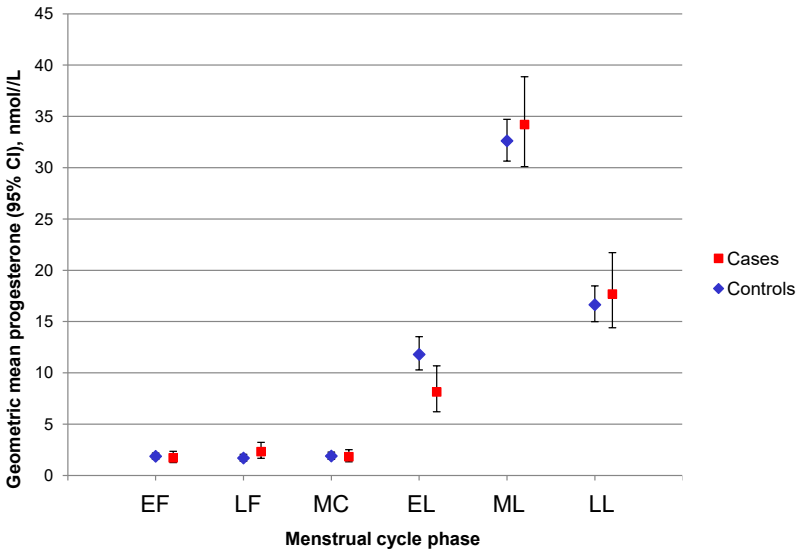
Geometric mean estradiol by phase of menstrual cycle



Adjusted for study, updated 1/11/12

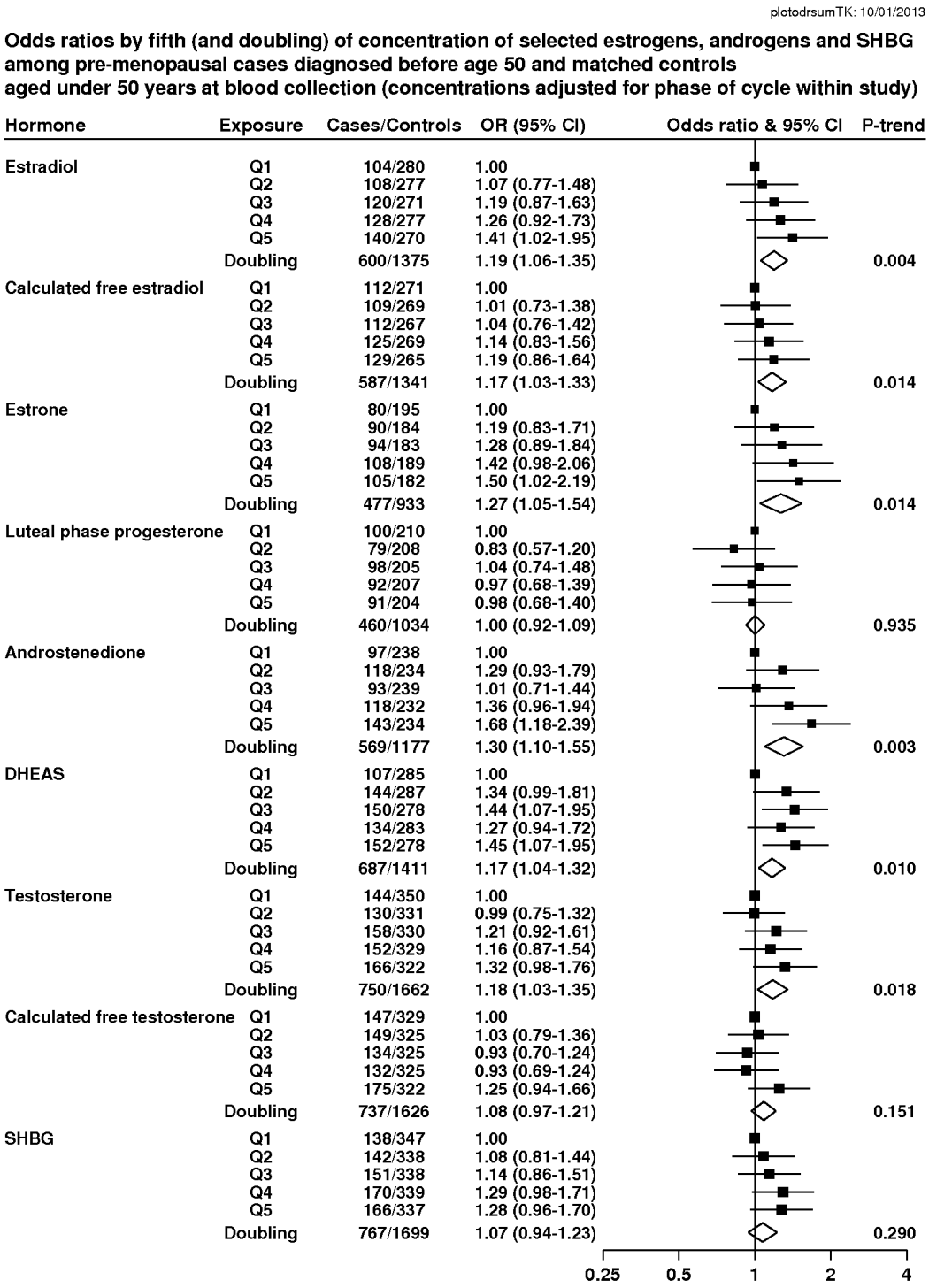
Figure 1b

Geometric mean progesterone by phase of menstrual cycle



Adjusted for study, updated 1/11/12

Figure 2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Odds ratios by fifth (and doubling) of concentration of selected estrogens, androgens and SHBG among pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Hormone Exposure Cases/Controls OR (95% CI) Odds ratio & 95% CI P-trend
Estradiol Q1 104/280 1.00
Q2 108/277 1.07 (0.77-1.48)
Q3 120/271 1.19 (0.87-1.63)
Q4 128/277 1.26 (0.92-1.73)
Q5 140/270 1.41 (1.02-1.95)
Doubling 600/1375 1.19 (1.06-1.35) 0.004
Calculated free estradiol Q1 112/271 1.00
Q2 109/269 1.01 (0.73-1.38)
Q3 112/267 1.04 (0.76-1.42)
Q4 125/269 1.14 (0.83-1.56)
Q5 129/265 1.19 (0.86-1.64)
Doubling 587/1341 1.17 (1.03-1.33) 0.014
Estrone Q1 80/195 1.00
Q2 90/184 1.19 (0.83-1.71)
Q3 94/183 1.28 (0.89-1.84)
Q4 108/189 1.42 (0.98-2.06)
Q5 105/182 1.50 (1.02-2.19)
Doubling 477/933 1.27 (1.05-1.54) 0.014
Luteal phase progesterone Q1 100/210 1.00
Q2 79/208 0.83 (0.57-1.20)
Q3 98/205 1.04 (0.74-1.48)
Q4 92/207 0.97 (0.68-1.39)
Q5 91/204 0.98 (0.68-1.40)
Doubling 460/1034 1.00 (0.92-1.09) 0.935
Androstenedione Q1 97/238 1.00
Q2 118/234 1.29 (0.93-1.79)
Q3 93/239 1.01 (0.71-1.44)
Q4 118/232 1.36 (0.96-1.94)
Q5 143/234 1.68 (1.18-2.39)
Doubling 569/1177 1.30 (1.10-1.55) 0.003
DHEAS Q1 107/285 1.00
Q2 144/287 1.34 (0.99-1.81)
Q3 150/278 1.44 (1.07-1.95)
Q4 134/283 1.27 (0.94-1.72)
Q5 152/278 1.45 (1.07-1.95)
Doubling 687/1411 1.17 (1.04-1.32) 0.010
Testosterone Q1 144/350 1.00
Q2 130/331 0.99 (0.75-1.32)
Q3 158/330 1.21 (0.92-1.61)
Q4 152/329 1.16 (0.87-1.54)
Q5 166/322 1.32 (0.98-1.76)
Doubling 750/1662 1.18 (1.03-1.35) 0.018
Calculated free testosterone Q1 147/329 1.00
Q2 149/325 1.03 (0.79-1.36)
Q3 134/325 0.93 (0.70-1.24)
Q4 132/325 0.93 (0.69-1.24)
Q5 175/322 1.25 (0.94-1.66)
Doubling 737/1626 1.08 (0.97-1.21) 0.151
SHBG Q1 138/347 1.00
Q2 142/338 1.08 (0.81-1.44)
Q3 151/338 1.14 (0.86-1.51)
Q4 170/339 1.29 (0.98-1.71)
Q5 166/337 1.28 (0.96-1.70)
Doubling 767/1699 1.07 (0.94-1.23) 0.290
0.25
0.5
1
2
4

Figure 3

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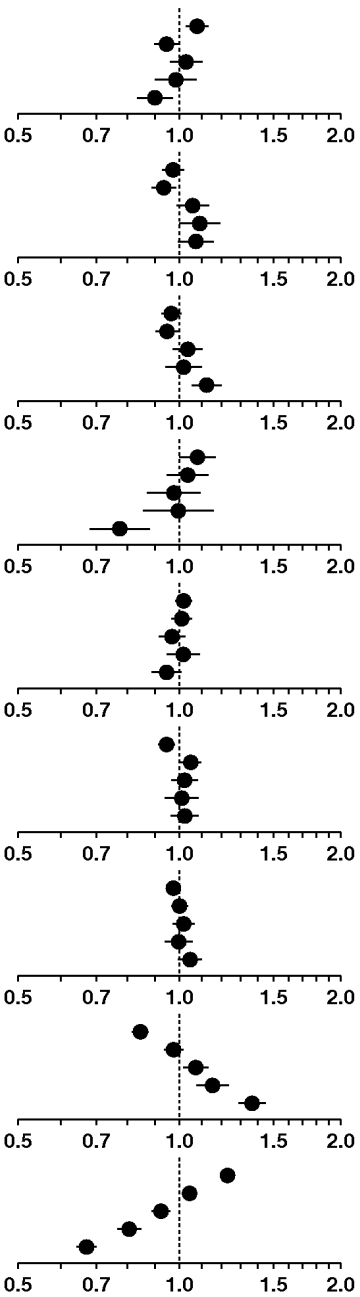
Mean concentration of selected hormones among premenopausal controls by categories of body mass index (kg/m^2), adjusted for study, age and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het (trend)	Relative mean$^+$ & 95% CI
Estradiol (pmol/L)				
<22.5 kg/m^2	829	250.9 (239.1-263.3)	<0.001	
22.5-24.9 kg/m^2	666	220.1 (208.6-232.2)	(0.001)	
25.0-27.4 kg/m^2	397	239.3 (223.2-256.5)		
27.5-29.9 kg/m^2	245	229.1 (209.7-250.3)		
≥30.0 kg/m^2	325	209.3 (193.8-226.1)		
Calculated free estradiol (pmol/L)				
<22.5 kg/m^2	821	2.86 (2.73-3.00)	0.002	
22.5-24.9 kg/m^2	661	2.75 (2.61-2.90)	(0.001)	
25.0-27.4 kg/m^2	391	3.12 (2.91-3.34)		
27.5-29.9 kg/m^2	241	3.21 (2.94-3.51)		
≥30.0 kg/m^2	321	3.16 (2.93-3.41)		
Estrone (pmol/L)				
<22.5 kg/m^2	501	202 (194-211)	<0.001	
22.5-24.9 kg/m^2	360	198 (189-208)	(<0.001)	
25.0-27.4 kg/m^2	214	217 (203-231)		
27.5-29.9 kg/m^2	144	213 (197-230)		
≥30.0 kg/m^2	222	235 (221-250)		
Luteal phase progesterone (nmol/L)				
<22.5 kg/m^2	691	29.81 (27.57-32.23)	0.001$^@$	
22.5-24.9 kg/m^2	529	28.58 (26.15-31.24)	(<0.001)	
25.0-27.4 kg/m^2	317	26.93 (24.00-30.22)		
27.5-29.9 kg/m^2	185	27.45 (23.62-31.91)		
≥30.0 kg/m^2	252	21.34 (18.75-24.30)		
Androstenedione (nmol/L)				
<22.5 kg/m^2	678	4.33 (4.18-4.49)	0.244	
22.5-24.9 kg/m^2	458	4.30 (4.12-4.49)	(0.069)	
25.0-27.4 kg/m^2	262	4.12 (3.89-4.37)		
27.5-29.9 kg/m^2	167	4.33 (4.03-4.65)		
≥30.0 kg/m^2	213	4.03 (3.78-4.29)		
DHEAS (nmol/L)				
<22.5 kg/m^2	917	2706 (2609-2807)	0.005	
22.5-24.9 kg/m^2	636	3003 (2876-3137)	(0.029)	
25.0-27.4 kg/m^2	366	2924 (2761-3097)		
27.5-29.9 kg/m^2	236	2889 (2689-3103)		
≥30.0 kg/m^2	343	2928 (2758-3108)		
Testosterone (nmol/L)				
<22.5 kg/m^2	1148	0.94 (0.91-0.97)	0.171	
22.5-24.9 kg/m^2	859	0.97 (0.93-1.00)	(0.023)	
25.0-27.4 kg/m^2	499	0.98 (0.94-1.03)		
27.5-29.9 kg/m^2	305	0.96 (0.91-1.02)		
≥30.0 kg/m^2	433	1.01 (0.96-1.06)		
Calculated free testosterone (pmol/L)				
<22.5 kg/m^2	1141	10.2 (9.9-10.6)	<0.001	
22.5-24.9 kg/m^2	855	11.8 (11.3-12.3)	(<0.001)	
25.0-27.4 kg/m^2	496	13.0 (12.3-13.7)		
27.5-29.9 kg/m^2	301	14.0 (13.0-15.0)		
≥30.0 kg/m^2	430	16.6 (15.6-17.6)		
SHBG (nmol/L)				
<22.5 kg/m^2	1154	67.9 (66.2-69.7)	<0.001$^@$	
22.5-24.9 kg/m^2	863	57.7 (56.0-59.5)	(<0.001)	
25.0-27.4 kg/m^2	501	51.0 (49.1-53.1)		
27.5-29.9 kg/m^2	303	44.5 (42.3-46.8)		
≥30.0 kg/m^2	432	37.0 (35.5-38.7)		



* means are geometric means

$^+$ means are scaled to the overall mean concentration (dotted line)

$^@$ indicates significant interaction with study (P<0.05)

Supplementary appendix

Sex hormones and breast cancer risk in premenopausal women: collaborative reanalysis of seven prospective studies

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Table of Contents

Web Table 1. Assay methods

Study	Estradiol		Estrone		Progesterone		Androstenedione		Testosterone		DHEAS		SHBG	
	Assay	CV %	Assay	CV%	Assay	CV %	Assay	CV %	Assay	CV %	Assay	CV%	Assay	CV %
CLUE I, USA	RIA, in-house with extraction	3.6/7.1	RIA, in-house with extraction	4.6/9.9	RIA (ICN)	2.9/7.7	RIA (in-house with extraction)	8.6/10.0			RIA (Wien Laboratories)	2.7	RIA (DPC)	8.0/10.9
Columbia, USA	RIA, in-house with extraction	8.5							RIA (in-house with extraction)	11.2			CIA (Siemens)	7.0
EPIC, Europe	RIA (DiaSorin)	3.2/7.2	RIA (DSL)	4.9/12.6	RIA (Immunotech)	7.4/9.4	RIA (DSL)	5.8/18.9	RIA (Immunotech)	8.4/15.3	RIA (Immunotech)	6.1/12.4	IRMA (Cis-Bio)	6.7/16.5
Guernsey, UK	RIA (DPC)	13.5/2.3			RIA (DPC)	7.8/15.8			RIA (IDS)	7.0/4.5			IRMA (in-house)	5.3/4.1
Nurses' Health Study II phase 1, USA	RIA with extraction (Quest Diagnostics)	-/<14	RIA with extraction (Quest Diagnostics)	-/<14	CIA (Diagnostic Products)	3/17	RIA (DSL)	-/<14	RIA with extraction (Quest Diagnostics)	-/9	CIA (DPC)	/≤12	CIA (Diagnostic Products)	-/<14
Nurses' Health Study II phase 2, USA	LCMS (Mayo Clinic)	-/≤13	LCMS (Mayo Clinic)	-/≤10	CIA (Diagnostic Products; Abbott Diagnostics)				LCMS (Mayo Clinic)	-/≤14	CIA (Diagnostic Products; Siemens)	-/≤11	CIA (Diagnostic Products; Abbott Diagnostics)	-/<14
NYU WHS, USA							RIA (DSL)	7.8/13.5	RIA (Immunotech)	8.7/15.8	RIA (Immunotech)	5.4/14.7	IRMA (Cis-Bio)	5.6/13.5
ORDET, Italy	RIA (Orion Diagnostica)	5.9/7.4[1]			RIA (Orion Diagnostica)	8.7/10.6	RIA (ICN Biomedical)	2.7/4.8	RIA (Orion Diagnostica)	4.6/9.1	RIA (Orion Diagnostica)	7.0/13.8	IRMA (Orion Diagnostica)	3.5/9.6

CV% = percentage coefficient of variation, intra-assay/inter-assay.

Assay abbreviations: CIA = chemiluminescent immunoassay; DSL = Diagnostic Systems Laboratories; DPC = Diagnostic Products Corporation; ECIA = electrochemiluminescence immunoassay; ICN = ICN Biomedicals Inc.; IRMA = Immunoradiometric assay; LCMS = liquid chromatography-tandem mass spectrometry; RIA = Radioimmunoassay.

Study abbreviations: CLUE I = Washington County, MD Study "Give us a clue to cancer and heart disease"; EPIC = European Prospective Investigation into Cancer and Nutrition; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors.

[1]CVs for high oestradiol concentration samples, CVs for low oestradiol were 14.0/16.4.

Web Table 2. Participant characteristics by study and case-control status

Study		Number[1]	Time to diagnosis (years)[2]	Age (years)	Age at menarche (years)	Nulliparous, n (%)	Age at first FTP (years)	BMI (kg/m²)	Family history of breast cancer, n (%)	Current smokers, n (%)	Alcohol (g/d)	Previous use of hormonal contraceptives, n (%)
CLUE I, USA	Cases	21	9 (4-12)	35.6 (5.3)	-	0 (0.0)	20.3 (2.0)	-	-	8 (38.1)	-	4 (19.0)
	Controls	42	-	35.8 (5.3)	-	2 (5.9)	20.4 (3.5)	-	-	9 (21.4)	-	6 (14.3)
Columbia, USA	Cases	13	3 (0-9)	39.5 (5.7)	12.7 (1.8)	4 (30.8)	22.1 (5.4)	26.6 (5.6)	3 (23.1)	3 (23.1)	-	-
	Controls	24	-	39.9 (3.5)	13.0 (1.4)	0 (0.0)	22.2 (3.5)	23.9 (4.4)	3 (13.0)	7 (29.2)	-	-
EPIC, Europe	Cases	206	2 (1-4)	42.2 (3.7)	12.7 (1.5)	30 (15.8)	25.8 (4.1)	24.6 (4.2)	-	51 (24.9)	8.0 (12.2)	126 (62.1)
	Controls	408	-	42.2 (3.7)	12.7 (1.5)	57 (15.2)	24.8 (3.9)	25.1 (4.6)	-	90 (22.4)	7.8 (12.7)	258 (64.0)
Guernsey, UK	Cases	32	5 (2-7)	38.9 (3.4)	13.0 (1.6)	4 (12.5)	24.7 (4.7)	24.0 (3.4)	5 (15.6)	1 (12.5)	-	28 (87.5)
	Controls	94	-	38.8 (3.2)	12.9 (1.5)	11 (11.7)	24.3 (4.1)	24.4 (3.9)	7 (7.4)	4 (16.7)	-	70 (74.5)
Nurses' Health Study II phase 1, USA	Cases	139	2 (1-3)	41.8 (3.6)	12.4 (1.4)	32 (23.4)	27.7 (4.4)	25.0 (5.3)	22 (15.8)	18 (12.9)	4.2 (7.4)	120 (86.3)
	Controls	268	-	41.6 (3.4)	12.5 (1.4)	53 (19.9)	26.6 (4.3)	25.0 (5.0)	24 (9.0)	17 (6.3)	3.7 (6.0)	225 (84.0)
Nurses' Health Study II phase 2, USA	Cases	105	8 (6-9)	38.1 (2.9)	12.5 (1.4)	31 (29.5)	27.0 (4.0)	23.5 (4.1)	18 (17.1)	11 (10.5)	3.2 (6.1)	79 (75.2)
	Controls	203	-	38.4 (2.9)	12.5 (1.5)	40 (19.8)	26.1 (3.8)	24.8 (5.5)	22 (10.8)	14 (6.9)	2.7 (4.9)	172 (84.7)
NYU WHS, USA	Cases	137	5 (2-7)	39.9 (3.3)	12.3 (1.4)	60 (46.9)	27.3 (6.4)	23.1 (4.5)	44 (32.1)	23 (18.9)	5.8 (12.6)	81 (66.9)
	Controls	258	-	39.9 (3.3)	12.4 (1.4)	107 (44.6)	26.3 (6.1)	23.7 (4.3)	45 (17.4)	41 (18.1)	7.8 (17.2)	154 (65.0)
ORDET, Italy	Cases	84	4 (1-8)	40.5 (3.3)	12.6 (1.6)	10 (11.9)	26.3 (4.2)	24.3 (4.4)	6 (7.2)	15 (17.9)	9.6 (14.2)	46 (55.4)
	Controls	336	-	40.6 (3.6)	12.5 (1.4)	48 (14.3)	25.0 (3.6)	24.0 (3.9)	28 (8.4)	89 (26.5)	9.1 (13.5)	165 (49.1)

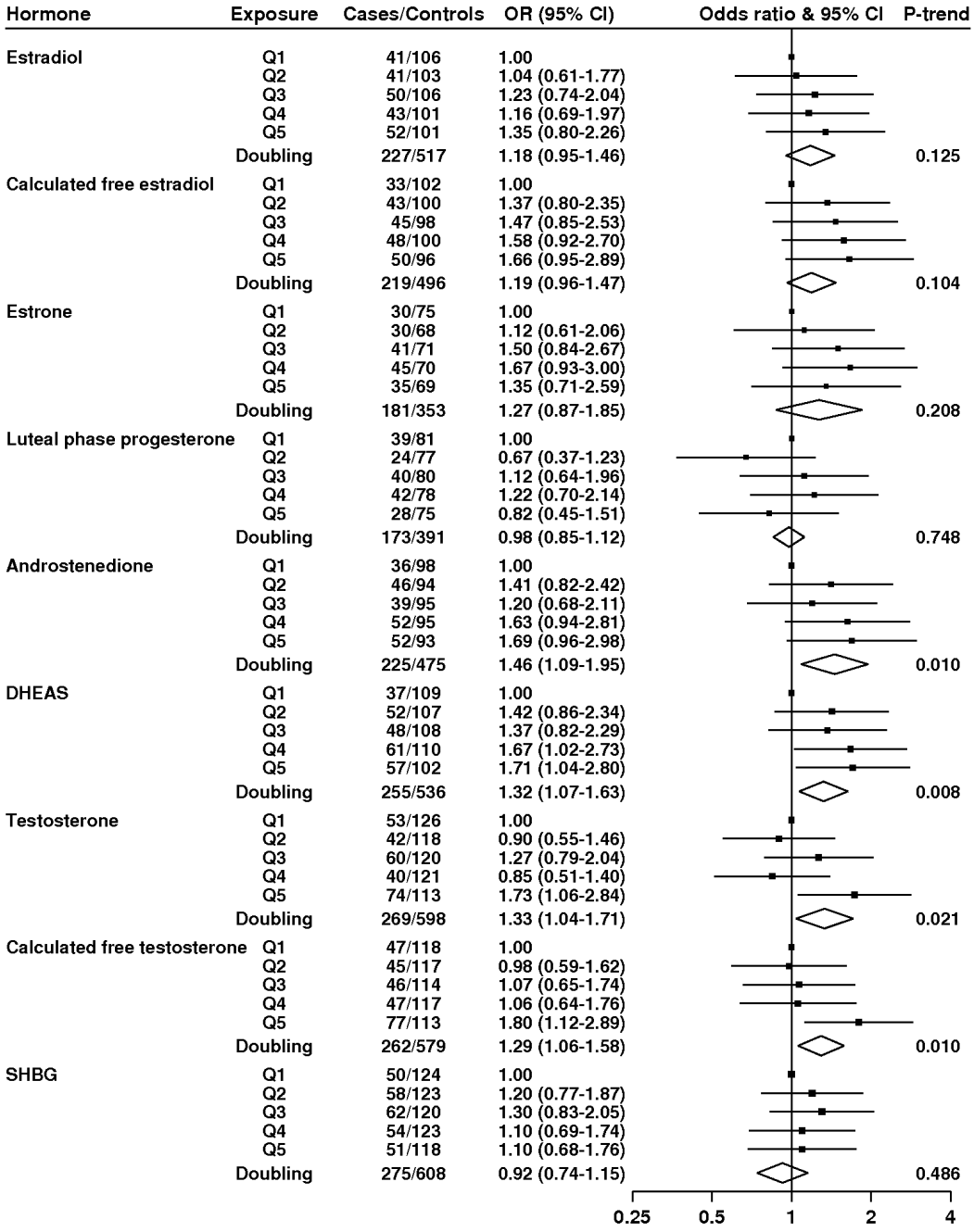
Values are mean (SD) unless otherwise indicated, percentages exclude women with missing values.
[1]Numbers are for women with known phase of cycle and values for oestradiol (except for NYU WHS where numbers are for women with values for testosterone).
[2]Median (inter-quartile range) time between blood collection and diagnosis for cases.

- indicates data not available or not applicable.
CLUE I = Washington County, MD Study “Give us a clue to cancer and heart disease”; EPIC = European Prospective Investigation into Cancer and Nutrition; NYU WHS = New York University Women’s Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors.

Web Figure 1

plotodrsumTK45: 16/01/2013

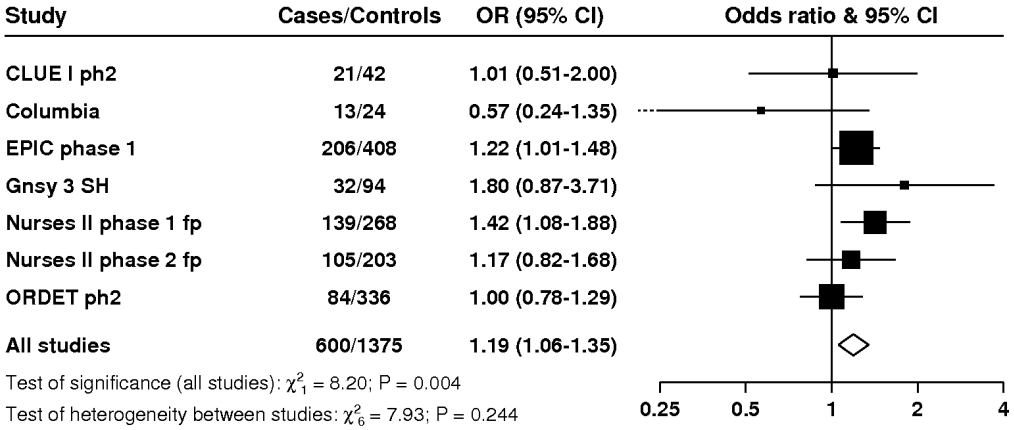
Odds ratios by fifth (and doubling) of concentration of selected estrogens, androgens and SHBG among pre-menopausal cases diagnosed before age 45 and matched controls aged under 45 years at blood collection (concentrations adjusted for phase of cycle within study)



Web Figure 2

plotodrtTK(C05): 17/10/2012

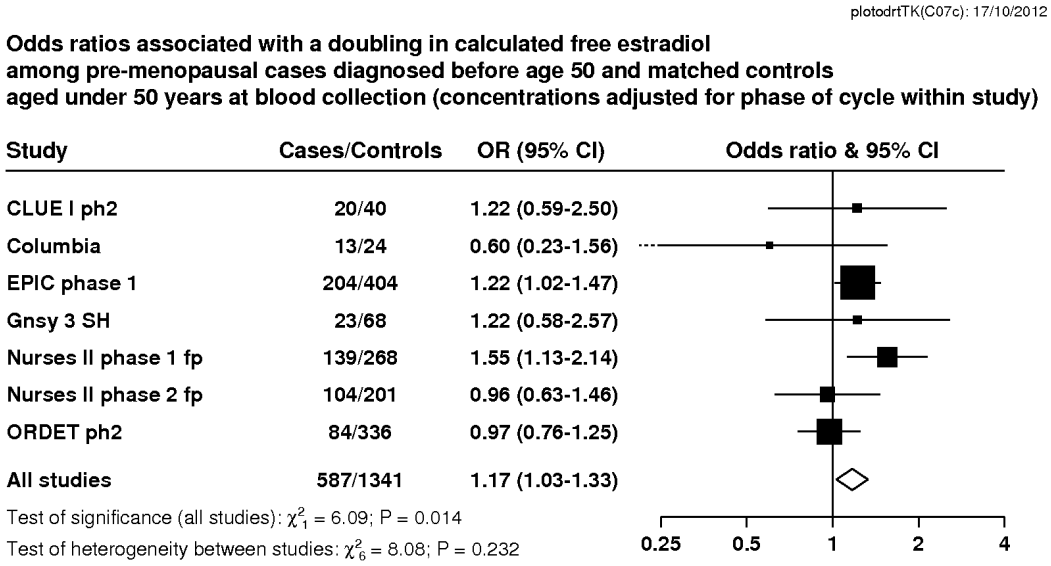
Odds ratios associated with a doubling in estradiol among pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)



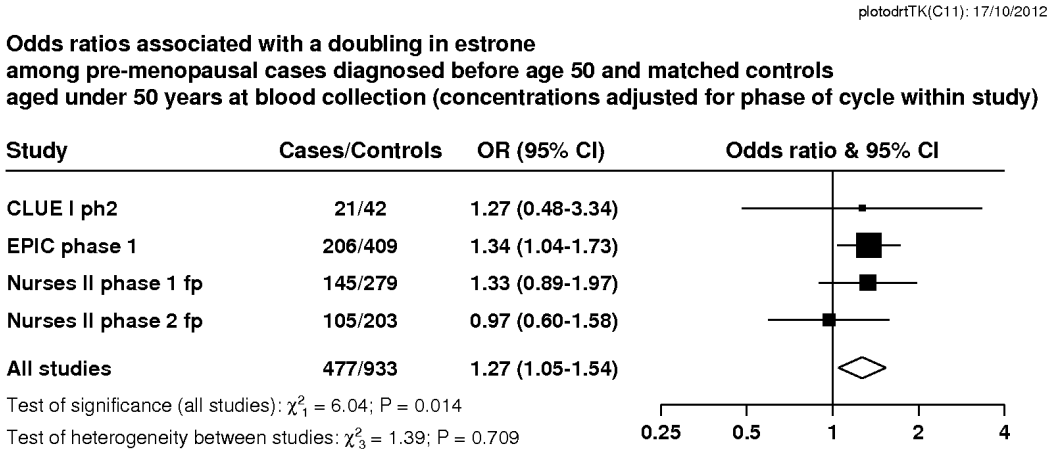
Test of significance (all studies): $\chi^2_1 = 8.20$; P = 0.004

Test of heterogeneity between studies: $\chi^2_6 = 7.93$; P = 0.244

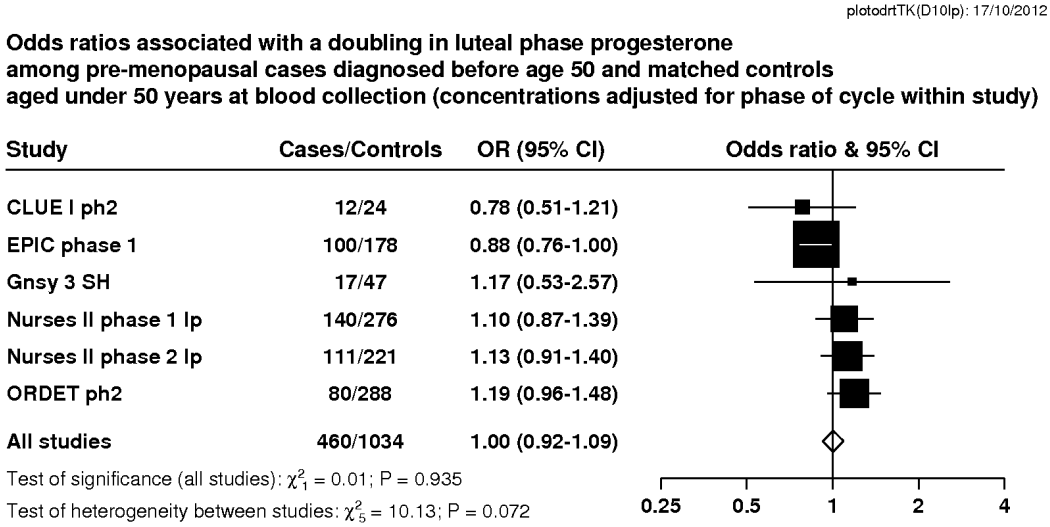
Web Figure 3


plotodrtTK(C07c): 17/10/2012
Odds ratios associated with a doubling in calculated free estradiol
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
CLUE I ph2
20/40
1.22 (0.59-2.50)
Columbia
13/24
0.60 (0.23-1.56)
EPIC phase 1
204/404
1.22 (1.02-1.47)
Gnsy 3 SH
23/68
1.22 (0.58-2.57)
Nurses II phase 1 fp
139/268
1.55 (1.13-2.14)
Nurses II phase 2 fp
104/201
0.96 (0.63-1.46)
ORDET ph2
84/336
0.97 (0.76-1.25)
All studies
587/1341
1.17 (1.03-1.33)
Test of significance (all studies): χ^2_1 = 6.09; P = 0.014
Test of heterogeneity between studies: χ^2_6 = 8.08; P = 0.232
0.25
0.5
1
2
4

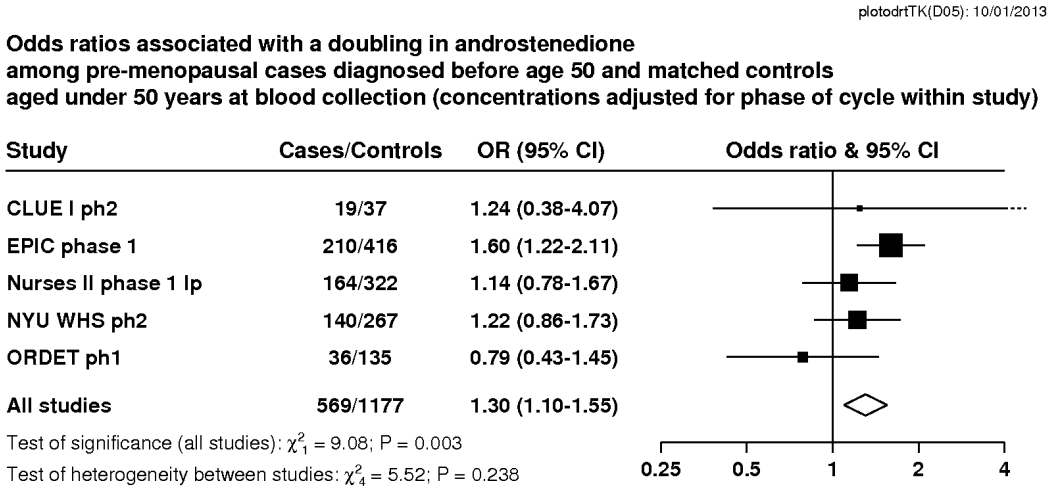
Web Figure 4


plotodrtTK(C11): 17/10/2012
Odds ratios associated with a doubling in estrone
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
CLUE I ph2
21/42
1.27 (0.48-3.34)
EPIC phase 1
206/409
1.34 (1.04-1.73)
Nurses II phase 1 fp
145/279
1.33 (0.89-1.97)
Nurses II phase 2 fp
105/203
0.97 (0.60-1.58)
All studies
477/933
1.27 (1.05-1.54)
Test of significance (all studies): χ^2_1 = 6.04; P = 0.014
Test of heterogeneity between studies: χ^2_3 = 1.39; P = 0.709
0.25
0.5
1
2
4

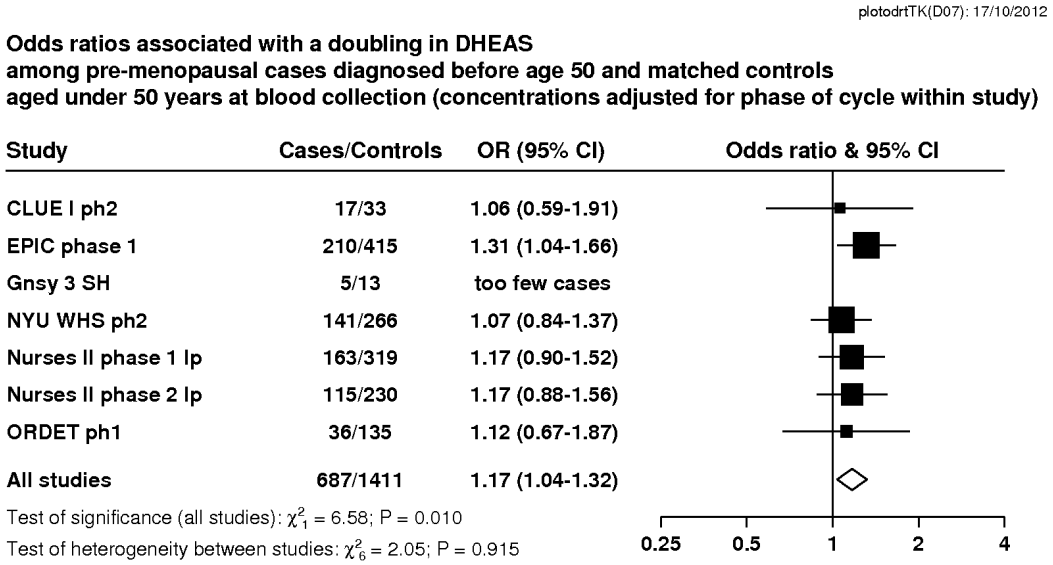
Web Figure 5


plotodrtTK(D10lp): 17/10/2012
Odds ratios associated with a doubling in luteal phase progesterone among pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
CLUE I ph2
12/24
0.78 (0.51-1.21)
EPIC phase 1
100/178
0.88 (0.76-1.00)
Gnsy 3 SH
17/47
1.17 (0.53-2.57)
Nurses II phase 1 lp
140/276
1.10 (0.87-1.39)
Nurses II phase 2 lp
111/221
1.13 (0.91-1.40)
ORDET ph2
80/288
1.19 (0.96-1.48)
All studies
460/1034
1.00 (0.92-1.09)
Test of significance (all studies): χ^2_1 = 0.01; P = 0.935
Test of heterogeneity between studies: χ^2_5 = 10.13; P = 0.072
0.25
0.5
1
2
4

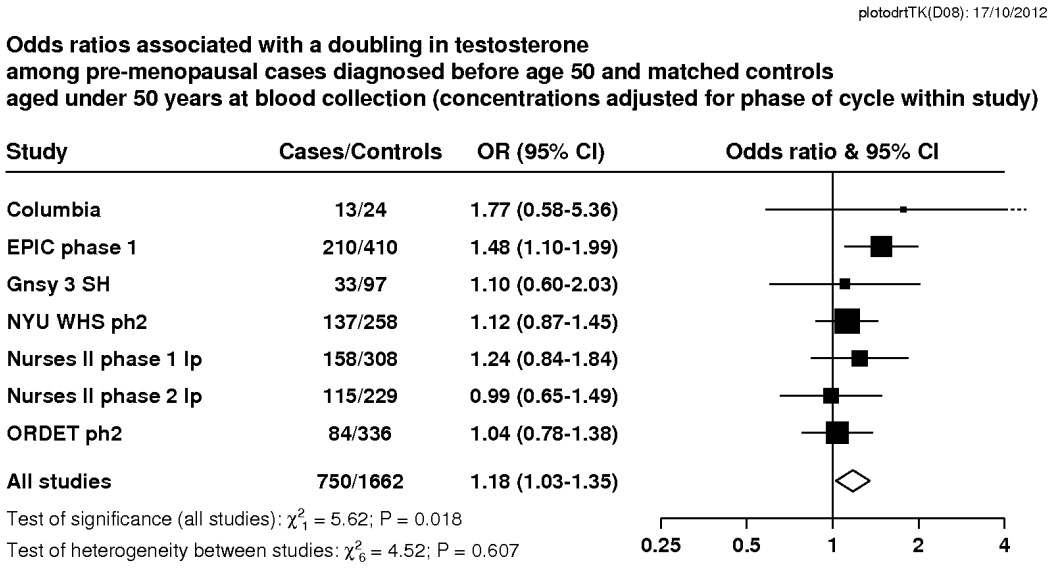
Web Figure 6


plotodrtTK(D05): 10/01/2013
Odds ratios associated with a doubling in androstenedione
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
CLUE I ph2
19/37
1.24 (0.38-4.07)
EPIC phase 1
210/416
1.60 (1.22-2.11)
Nurses II phase 1 Ip
164/322
1.14 (0.78-1.67)
NYU WHS ph2
140/267
1.22 (0.86-1.73)
ORDET ph1
36/135
0.79 (0.43-1.45)
All studies
569/1177
1.30 (1.10-1.55)
Test of significance (all studies): χ^2_1 = 9.08; P = 0.003
Test of heterogeneity between studies: χ^2_4 = 5.52; P = 0.238
0.25
0.5
1
2
4

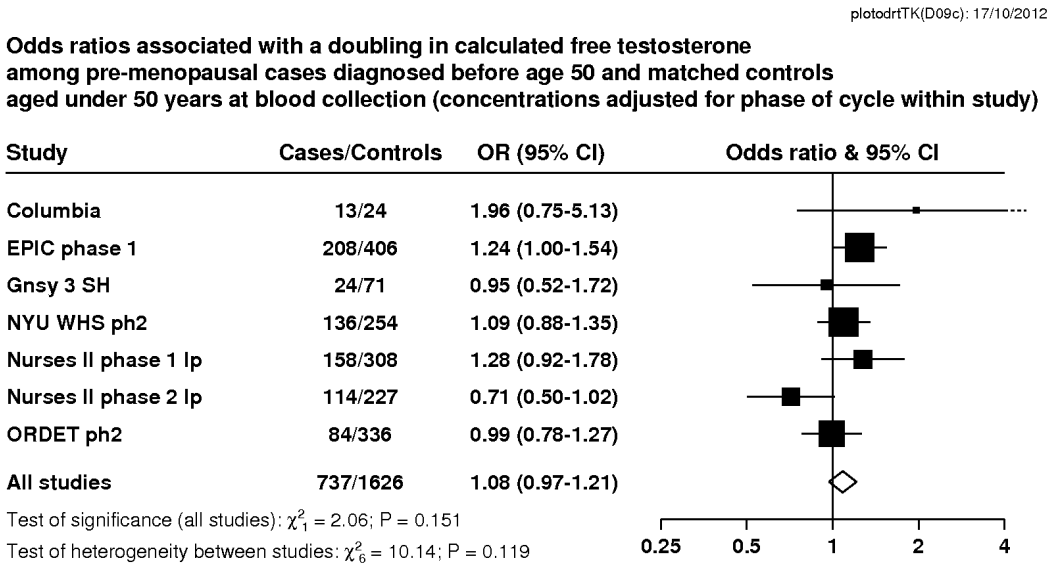
Web Figure 7


plotodrtTK(D07): 17/10/2012
Odds ratios associated with a doubling in DHEAS
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
CLUE I ph2
17/33
1.06 (0.59-1.91)
EPIC phase 1
210/415
1.31 (1.04-1.66)
Gnsy 3 SH
5/13
too few cases
NYU WHS ph2
141/266
1.07 (0.84-1.37)
Nurses II phase 1 Ip
163/319
1.17 (0.90-1.52)
Nurses II phase 2 Ip
115/230
1.17 (0.88-1.56)
ORDET ph1
36/135
1.12 (0.67-1.87)
All studies
687/1411
1.17 (1.04-1.32)
Test of significance (all studies): χ^2_1 = 6.58; P = 0.010
Test of heterogeneity between studies: χ^2_6 = 2.05; P = 0.915
0.25
0.5
1
2
4

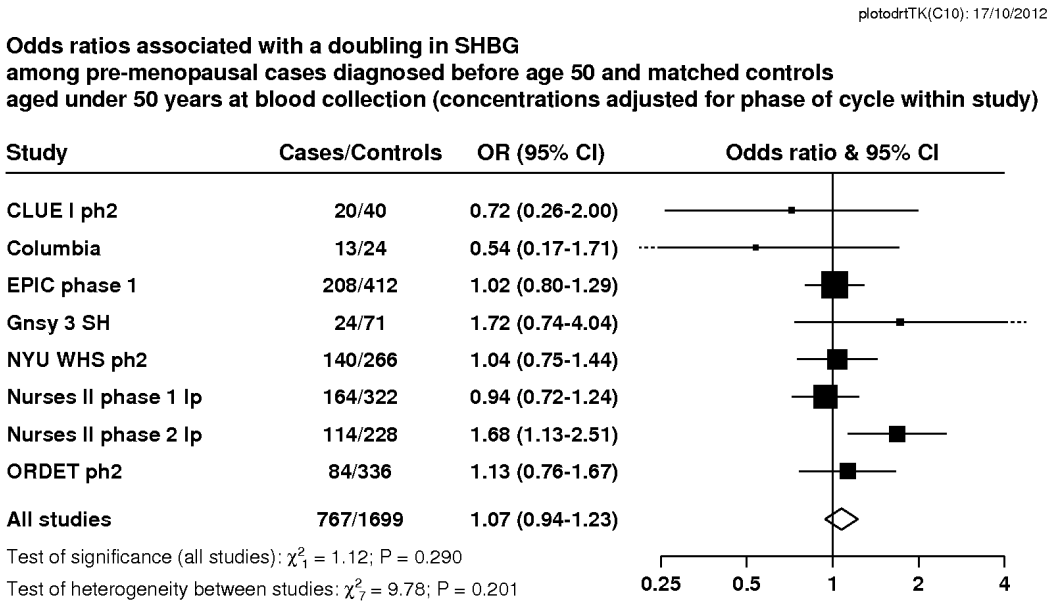
Web Figure 8


plotodrtTK(D08): 17/10/2012
Odds ratios associated with a doubling in testosterone
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
Columbia 13/24 1.77 (0.58-5.36)
EPIC phase 1 210/410 1.48 (1.10-1.99)
Gnsy 3 SH 33/97 1.10 (0.60-2.03)
NYU WHS ph2 137/258 1.12 (0.87-1.45)
Nurses II phase 1 lp 158/308 1.24 (0.84-1.84)
Nurses II phase 2 lp 115/229 0.99 (0.65-1.49)
ORDET ph2 84/336 1.04 (0.78-1.38)
All studies 750/1662 1.18 (1.03-1.35)
Test of significance (all studies): χ^2_1 = 5.62; P = 0.018
Test of heterogeneity between studies: χ^2_6 = 4.52; P = 0.607
0.25
0.5
1
2
4

Web Figure 9


plotodrtTK(D09c): 17/10/2012
Odds ratios associated with a doubling in calculated free testosterone
among pre-menopausal cases diagnosed before age 50 and matched controls
aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study)
Study
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
Columbia
13/24
1.96 (0.75-5.13)
EPIC phase 1
208/406
1.24 (1.00-1.54)
Gnsy 3 SH
24/71
0.95 (0.52-1.72)
NYU WHS ph2
136/254
1.09 (0.88-1.35)
Nurses II phase 1 lp
158/308
1.28 (0.92-1.78)
Nurses II phase 2 lp
114/227
0.71 (0.50-1.02)
ORDET ph2
84/336
0.99 (0.78-1.27)
All studies
737/1626
1.08 (0.97-1.21)
Test of significance (all studies): χ^2_1 = 2.06; P = 0.151
Test of heterogeneity between studies: χ^2_6 = 10.14; P = 0.119
0.25
0.5
1
2
4

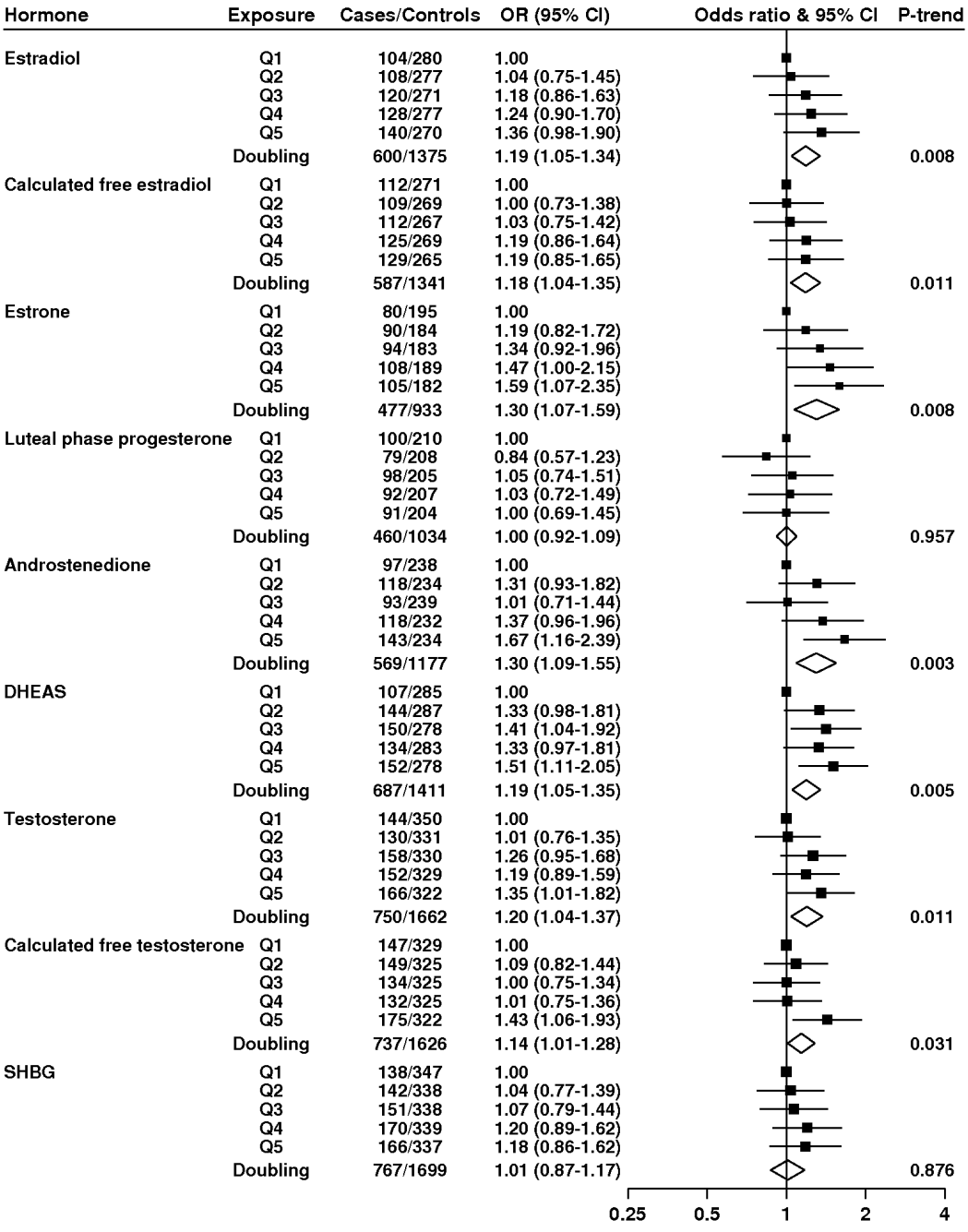
Web Figure 10



Web Figure 11

plotodrsumTKa: 10/01/2013

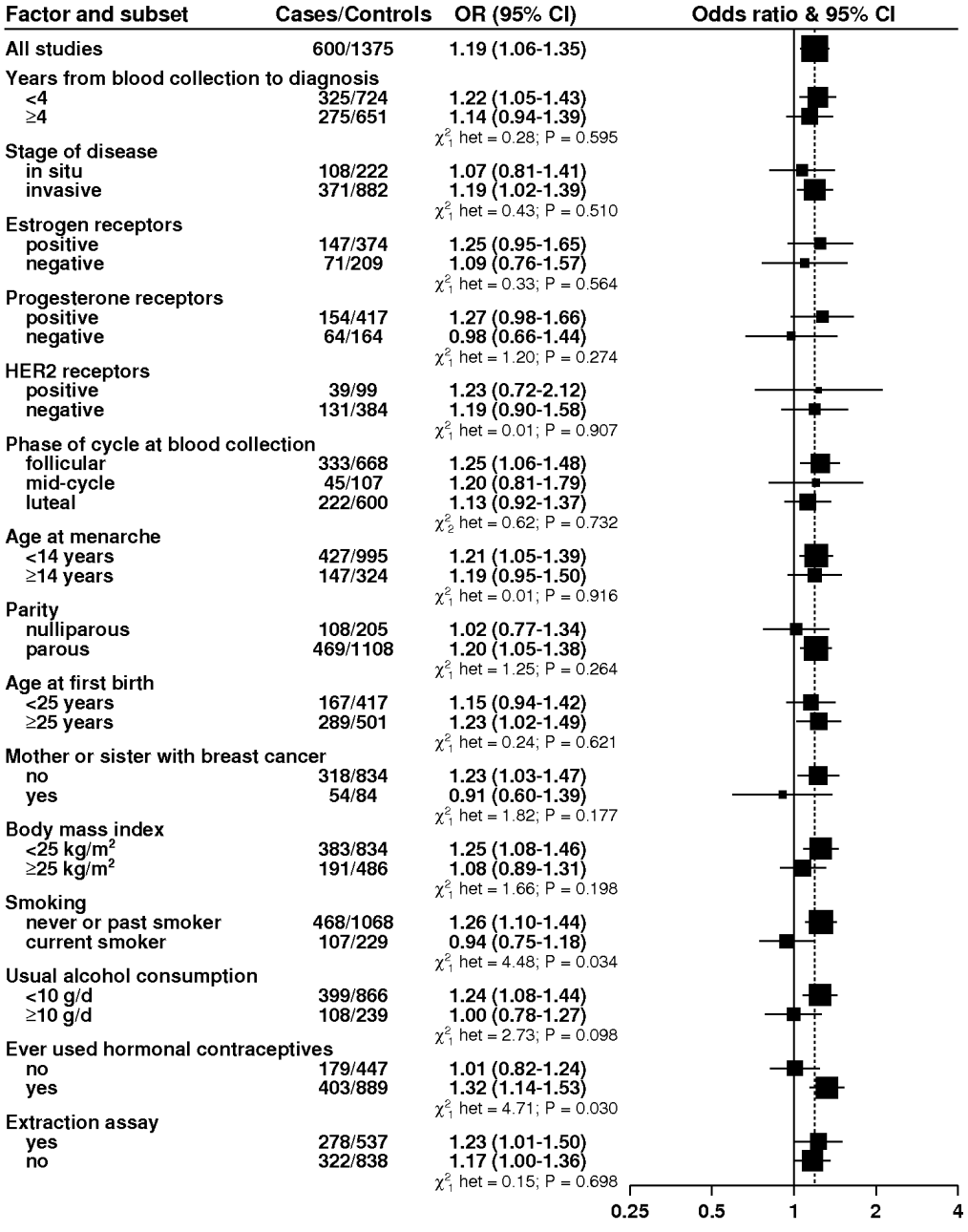
Odds ratios by fifth (and doubling) of concentration of selected estrogens, androgens and SHBG among pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection (concentrations adjusted for phase of cycle within study) adjusting for age at menarche, parity, age at first full-term pregnancy and BMI



Web Figure 12

plotodrsTK(C05): 17/10/2012

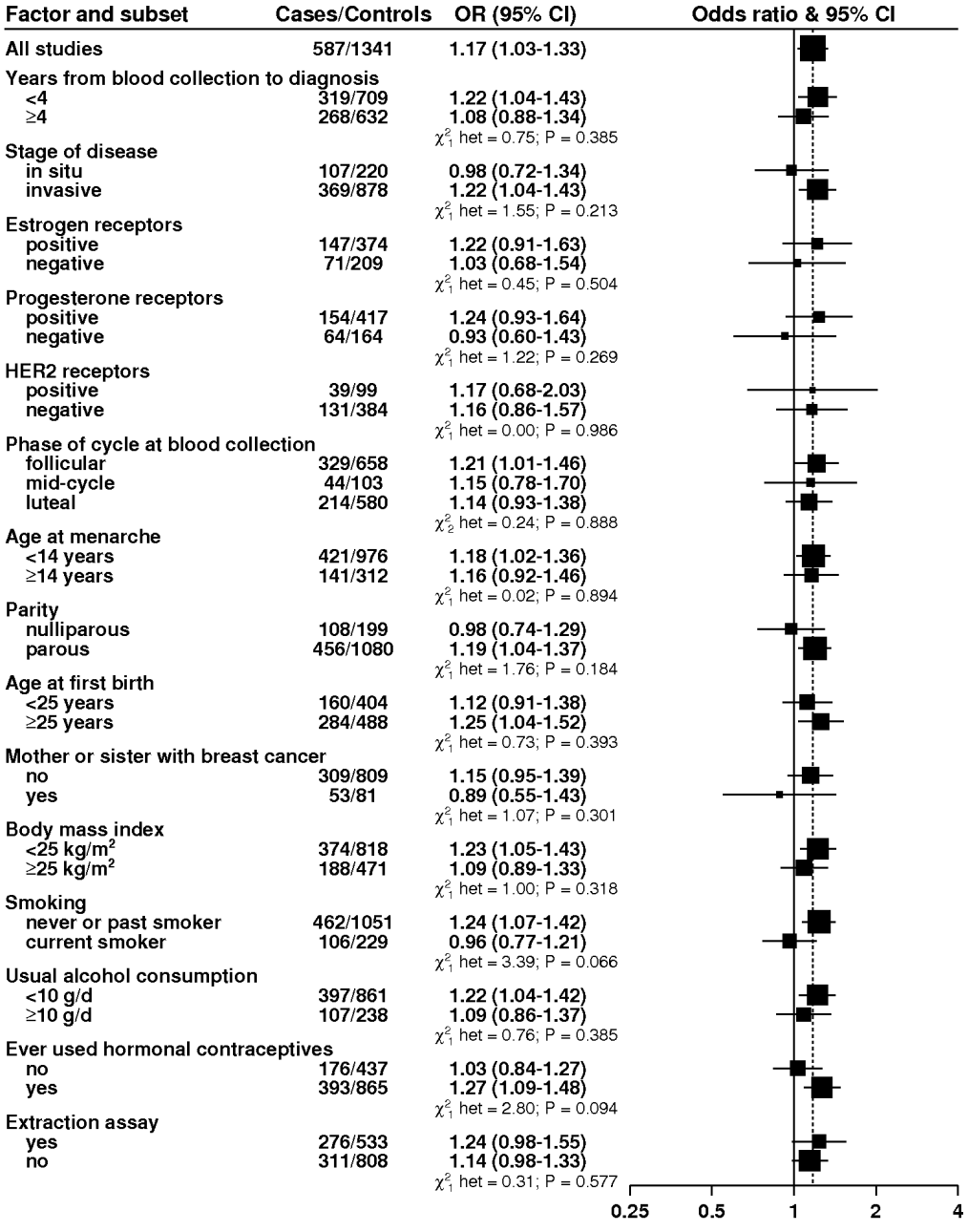
Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in estradiol, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



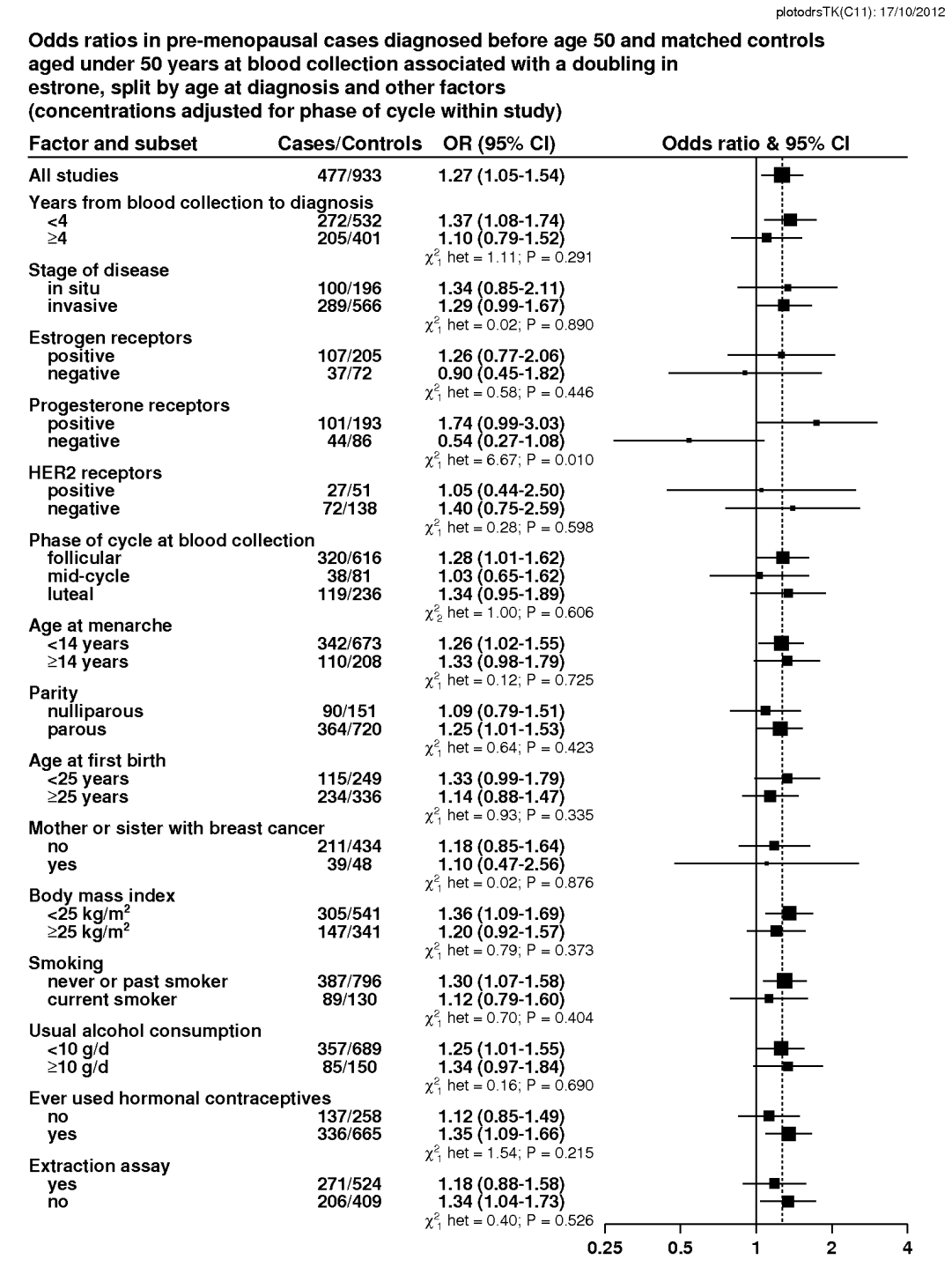
Web Figure 13

plotodrsTK(C07c): 17/10/2012

Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in calculated free estradiol, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



Web Figure 14

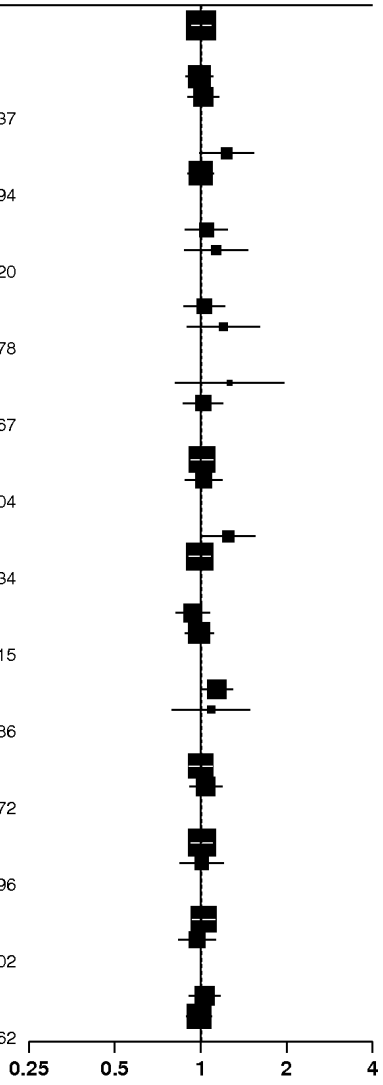


Web Figure 15

plotodrsTK(D10lp): 01/11/2012

Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in luteal phase progesterone, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)

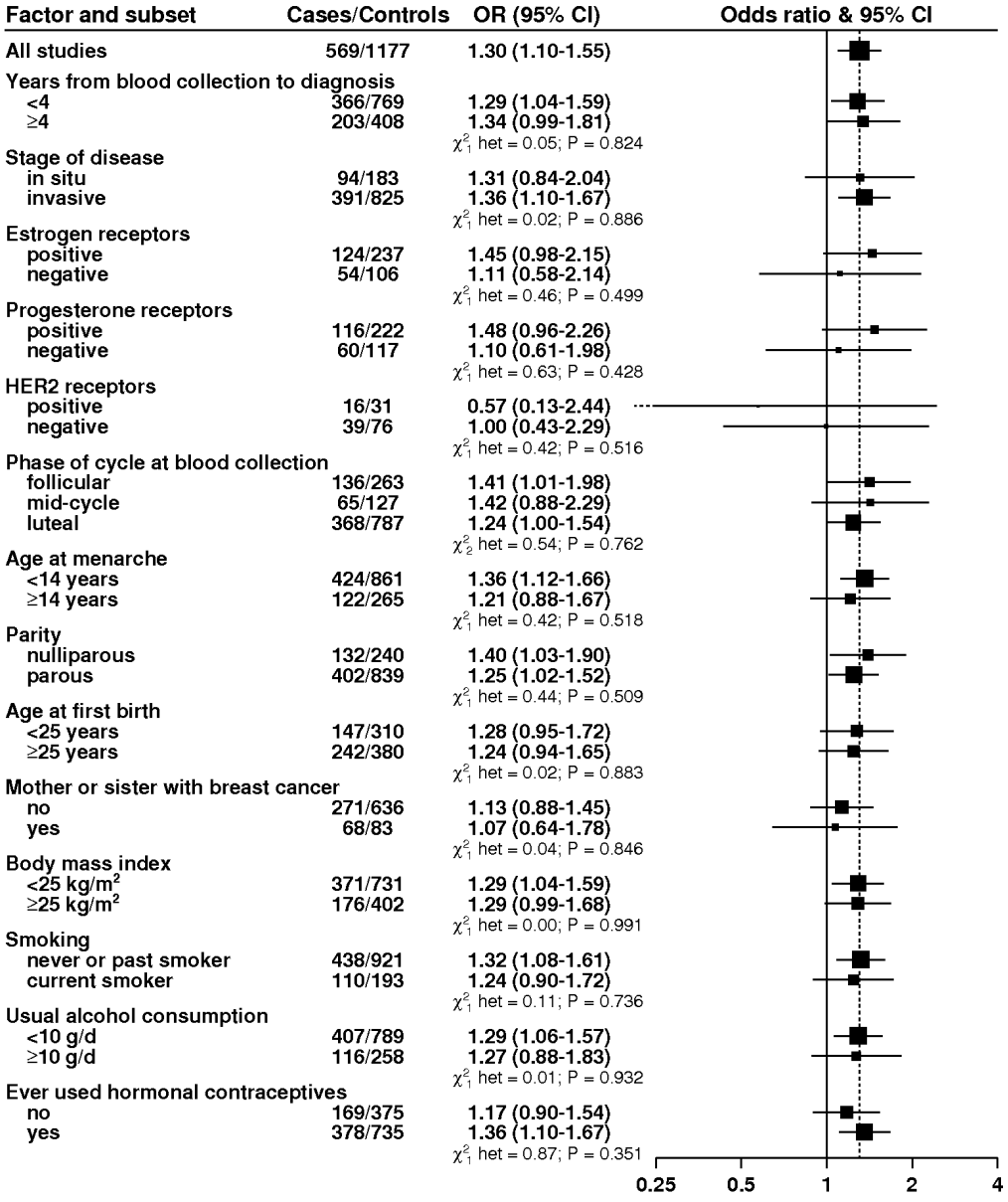
Factor and subset	Cases/Controls	OR (95% CI)
All studies	460/1034	1.00 (0.92-1.09)
Years from blood collection to diagnosis		
<4	231/500	0.99 (0.89-1.11)
≥4	229/534	1.02 (0.90-1.16)
		χ^2_1 het = 0.11; P = 0.737
Stage of disease		
in situ	99/207	1.23 (0.99-1.53)
invasive	284/668	1.00 (0.90-1.11)
		χ^2_1 het = 2.80; P = 0.094
Estrogen receptors		
positive	152/369	1.05 (0.88-1.24)
negative	67/184	1.13 (0.88-1.47)
		χ^2_1 het = 0.25; P = 0.620
Progesterone receptors		
positive	158/402	1.03 (0.87-1.22)
negative	61/150	1.20 (0.89-1.62)
		χ^2_1 het = 0.78; P = 0.378
HER2 receptors		
positive	36/90	1.26 (0.81-1.96)
negative	136/364	1.02 (0.87-1.20)
		χ^2_1 het = 0.81; P = 0.367
Age at menarche		
<14 years	334/776	1.01 (0.92-1.11)
≥14 years	109/221	1.02 (0.88-1.19)
		χ^2_1 het = 0.01; P = 0.904
Parity		
nulliparous	86/161	1.25 (1.01-1.55)
parous	357/835	0.99 (0.90-1.09)
		χ^2_1 het = 4.48; P = 0.034
Age at first birth		
<25 years	115/278	0.94 (0.82-1.07)
≥25 years	233/410	0.99 (0.88-1.11)
		χ^2_1 het = 0.42; P = 0.515
Mother or sister with breast cancer		
no	297/750	1.14 (1.00-1.30)
yes	50/77	1.09 (0.79-1.49)
		χ^2_1 het = 0.07; P = 0.786
Body mass index		
<25 kg/m^2	302/644	1.00 (0.90-1.10)
≥25 kg/m^2	142/354	1.04 (0.91-1.19)
		χ^2_1 het = 0.32; P = 0.572
Smoking		
never or past smoker	365/840	1.01 (0.92-1.11)
current smoker	79/152	1.01 (0.84-1.21)
		χ^2_1 het = 0.00; P = 0.996
Usual alcohol consumption		
<10 g/d	332/702	1.03 (0.93-1.13)
≥10 g/d	73/161	0.97 (0.83-1.13)
		χ^2_1 het = 0.45; P = 0.502
Ever used hormonal contraceptives		
no	131/314	1.03 (0.91-1.17)
yes	326/711	0.99 (0.89-1.09)
		χ^2_1 het = 0.34; P = 0.562



Web Figure 16

plotodrsTK(D05): 10/01/2013

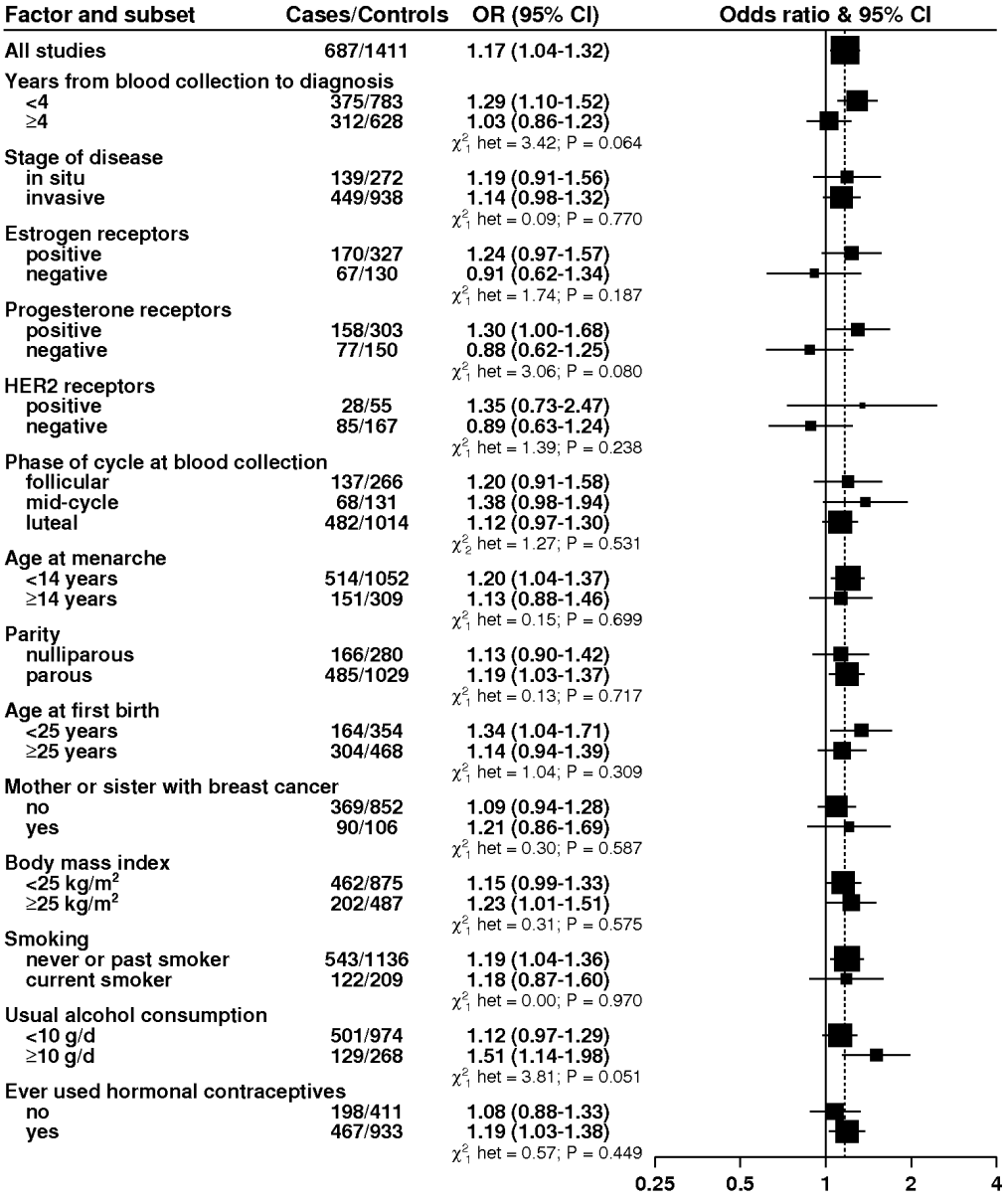
Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in androstenedione, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



Web Figure 17

plotodrsTK(D07): 01/11/2012

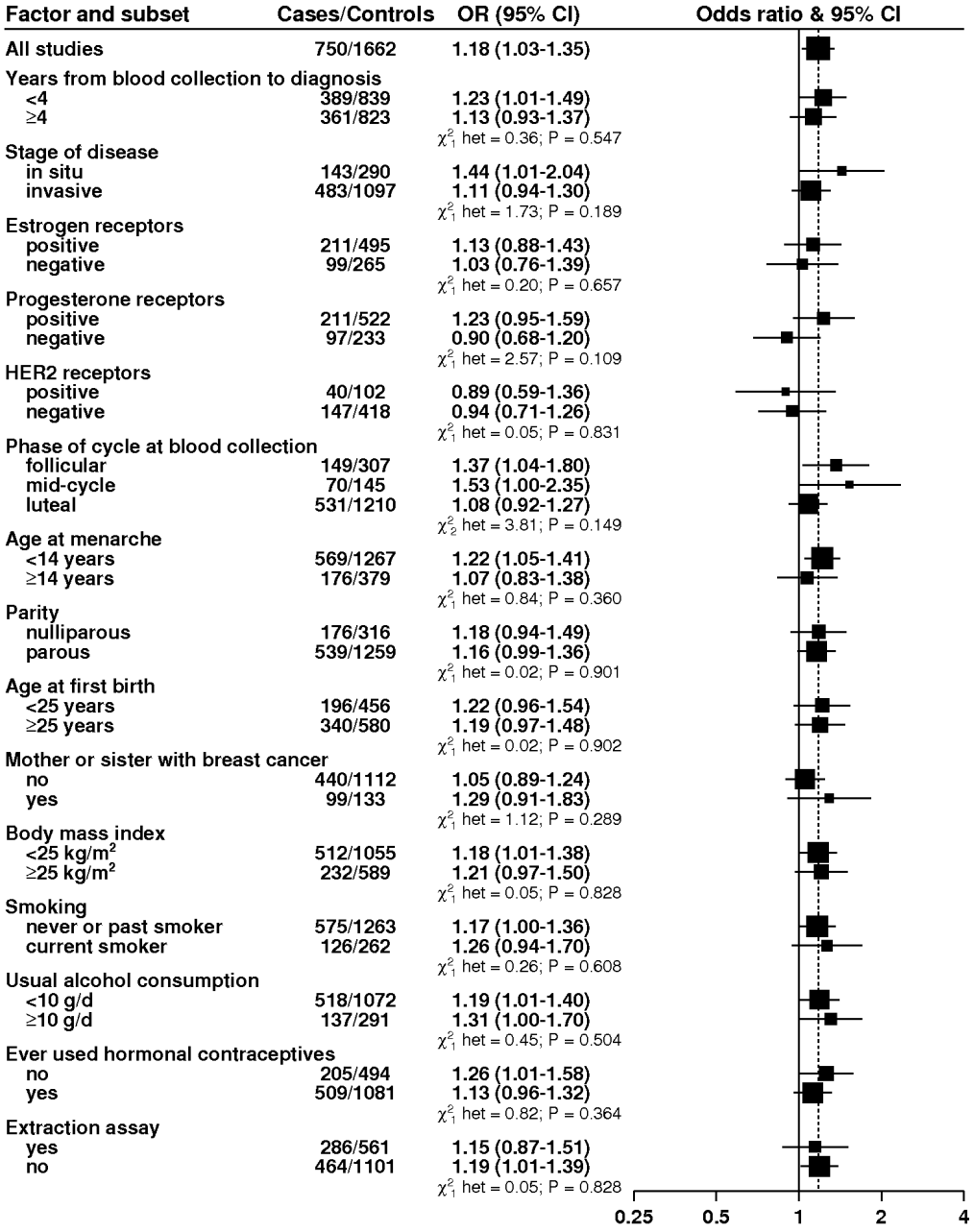
Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in DHEAS, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



Web Figure 18

plotodrsTK(D08): 17/10/2012

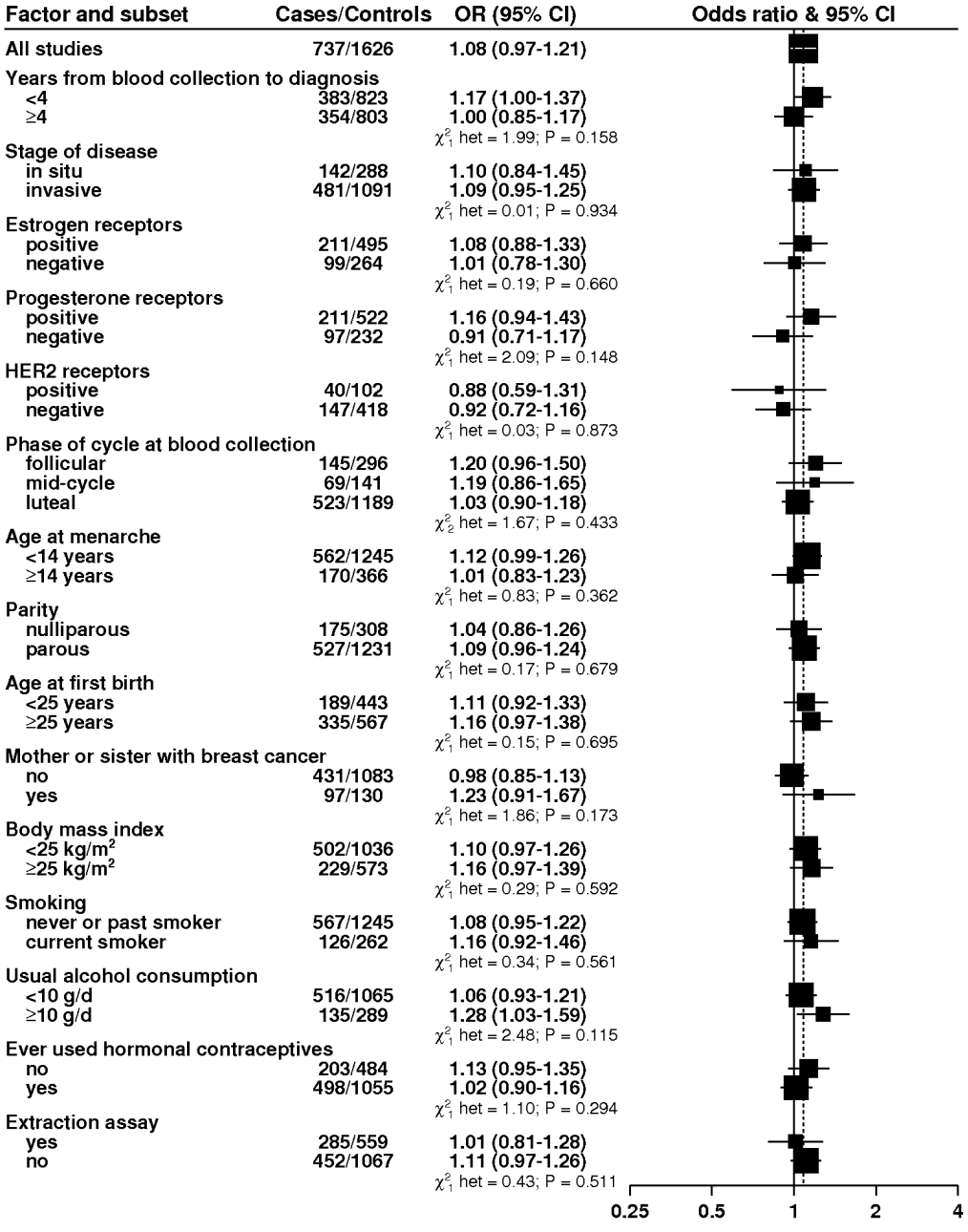
Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in testosterone, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



Web Figure 19

plotodrsTK(D09c): 17/10/2012

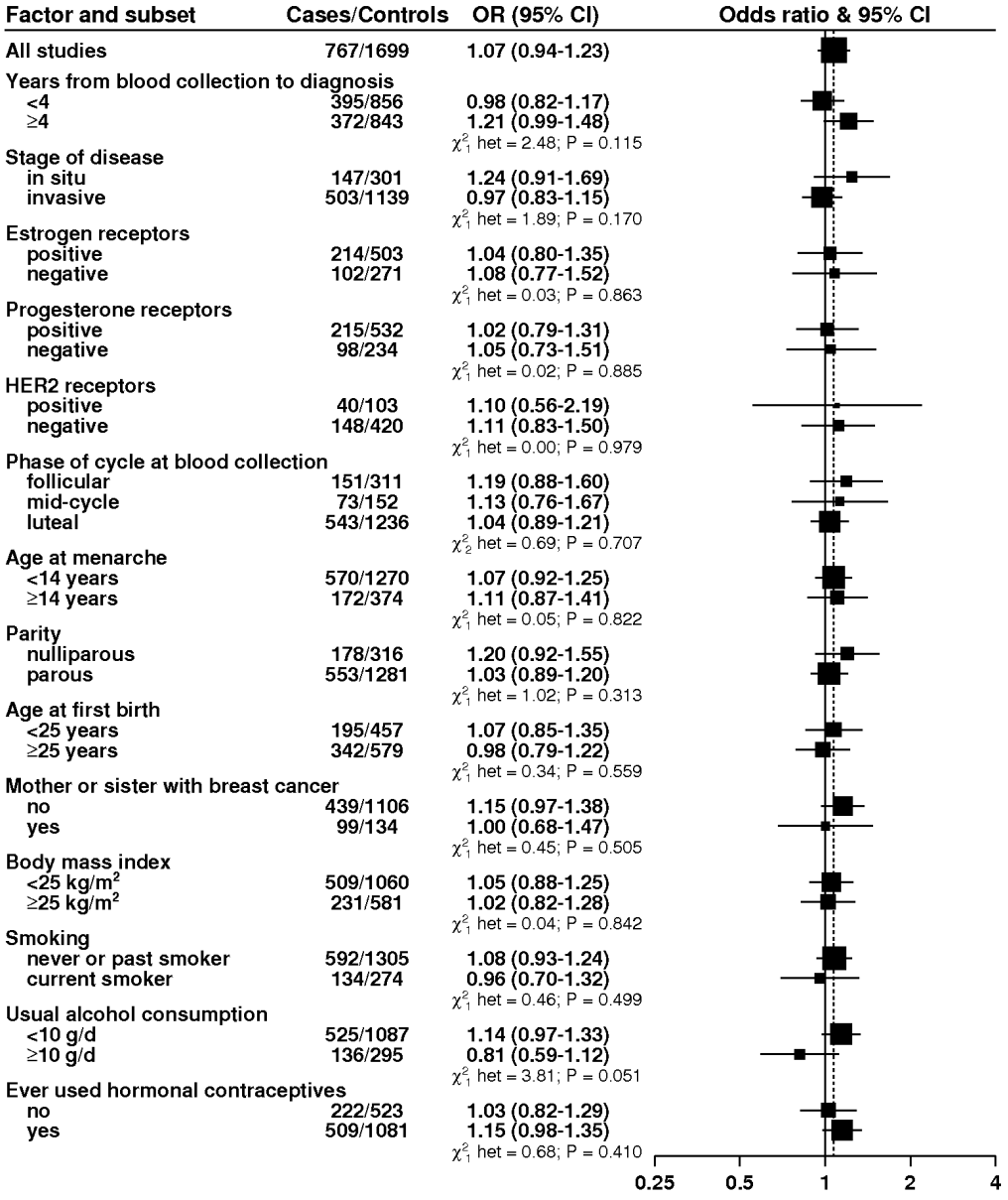
Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in calculated free testosterone, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)



Web Figure 20

plotodrsTK(C10): 01/11/2012

Odds ratios in pre-menopausal cases diagnosed before age 50 and matched controls aged under 50 years at blood collection associated with a doubling in SHBG, split by age at diagnosis and other factors (concentrations adjusted for phase of cycle within study)

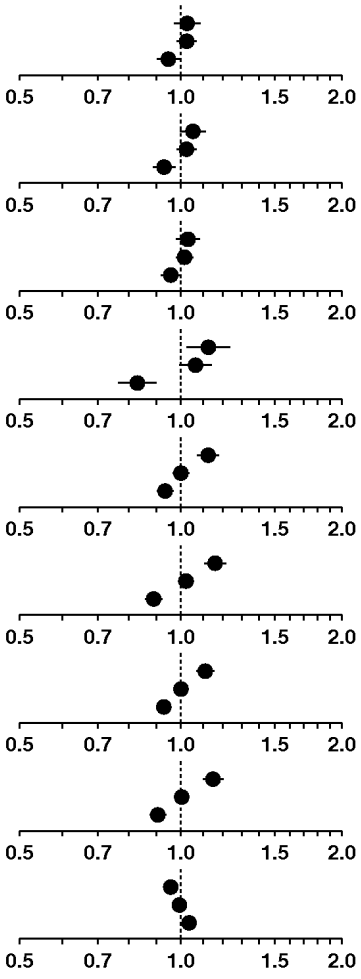


Web Figure 21

plotmhpreyTK(01): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of age at blood collection, adjusted for study, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het (trend)	Relative mean⁺ & 95% CI
Estradiol (pmol/L)				
under 40	637	239.1 (226.0-252.8)	0.033	
40-44	1046	238.6 (228.6-249.1)	(0.025)	
45-49	840	220.4 (210.0-231.4)		
Calculated free estradiol (pmol/L)				
under 40	630	3.10 (2.94-3.28)	0.002	
40-44	1041	3.02 (2.89-3.15)	(0.001)	
45-49	824	2.74 (2.61-2.87)		
Estrone (pmol/L)				
under 40	351	216 (205-227)	0.051	
40-44	640	213 (205-221)	(0.023)	
45-49	506	200 (192-209)		
Luteal phase progesterone (nmol/L)				
under 40	502	31.06 (28.30-34.10)	<0.001	
40-44	890	29.39 (27.43-31.50)	(<0.001)	
45-49	625	22.88 (21.05-24.87)		
Androstenedione (nmol/L)				
under 40	402	4.79 (4.57-5.02)	<0.001@	
40-44	710	4.26 (4.11-4.41)	(<0.001)	
45-49	718	3.98 (3.84-4.12)		
DHEAS (nmol/L)				
under 40	575	3315 (3164-3474)	<0.001@	
40-44	1045	2925 (2826-3026)	(<0.001)	
45-49	935	2544 (2453-2639)		
Testosterone (nmol/L)				
under 40	775	1.07 (1.03-1.11)	<0.001@	
40-44	1341	0.97 (0.94-1.00)	(<0.001)	
45-49	1154	0.90 (0.87-0.93)		
Calculated free testosterone (pmol/L)				
under 40	768	13.9 (13.3-14.5)	<0.001@	
40-44	1335	12.2 (11.8-12.6)	(<0.001)	
45-49	1145	11.0 (10.6-11.4)		
SHBG (nmol/L)				
under 40	803	52.9 (51.3-54.6)	0.001@	
40-44	1356	54.9 (53.6-56.2)	(<0.001)	
45-49	1163	57.3 (55.8-58.8)		



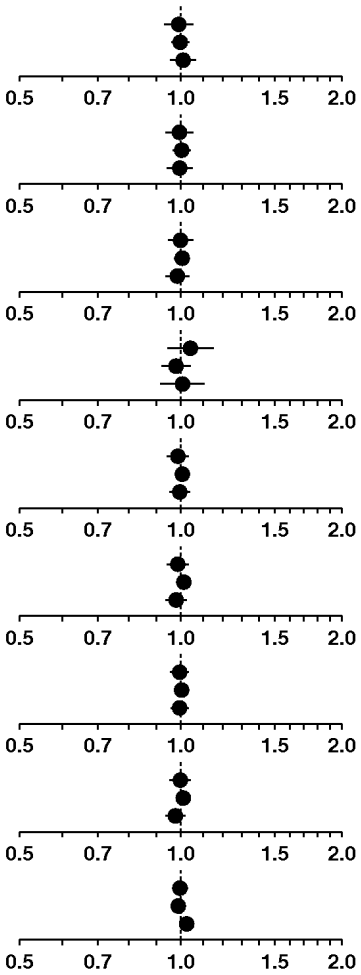
* means are geometric means
⁺ means are scaled to the overall mean concentration (dotted line)
@ indicates significant interaction with study (P<0.05)

Web Figure 22

plotmhpreyTK(09): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of age at menarche, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het (trend)
Estradiol (pmol/L)			
under 12	522	230.6 (216.9-245.2)	0.899
12-13	1303	232.1 (223.3-241.2)	(0.655)
14 and over	649	235.0 (222.3-248.3)	
Calculated free estradiol (pmol/L)			
under 12	516	2.93 (2.75-3.11)	0.949
12-13	1290	2.96 (2.84-3.07)	(0.996)
14 and over	641	2.93 (2.77-3.09)	
Estrone (pmol/L)			
under 12	313	209 (198-220)	0.799
12-13	796	210 (203-218)	(0.714)
14 and over	342	206 (196-217)	
Luteal phase progesterone (nmol/L)			
under 12	433	28.75 (26.03-31.75)	0.573@
12-13	1080	27.02 (25.39-28.76)	(0.649)
14 and over	474	27.79 (25.26-30.57)	
Androstenedione (nmol/L)			
under 12	397	4.20 (4.01-4.40)	0.771
12-13	944	4.29 (4.16-4.42)	(0.823)
14 and over	434	4.24 (4.05-4.43)	
DHEAS (nmol/L)			
under 12	553	2823 (2693-2959)	0.408
12-13	1373	2898 (2813-2985)	(0.822)
14 and over	583	2803 (2677-2935)	
Testosterone (nmol/L)			
under 12	709	0.96 (0.92-1.00)	0.909
12-13	1751	0.97 (0.95-0.99)	(0.978)
14 and over	798	0.96 (0.93-1.00)	
Calculated free testosterone (pmol/L)			
under 12	703	12.1 (11.6-12.7)	0.432
12-13	1743	12.2 (11.9-12.6)	(0.476)
14 and over	791	11.8 (11.3-12.4)	
SHBG (nmol/L)			
under 12	708	55.0 (53.2-56.9)	0.172
12-13	1762	54.7 (53.5-55.8)	(0.198)
14 and over	797	56.7 (54.9-58.5)	



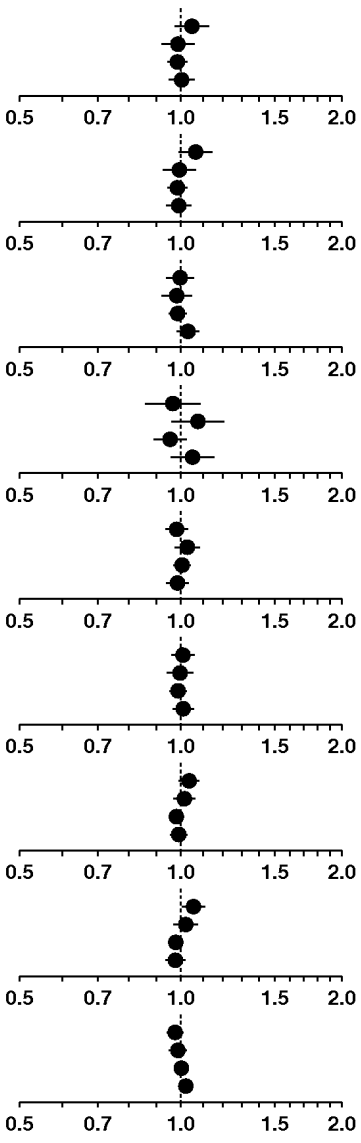
* means are geometric means
+ means are scaled to the overall mean concentration (dotted line)
@ indicates significant interaction with study (P<0.05)

Web Figure 23

plotmhpreyTK(17): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of number of full-term pregnancies, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het (trend)
Estradiol (pmol/L)			
none	359	244.1 (226.6-262.8)	0.534
one	391	229.7 (214.1-246.5)	(0.429)
two	1068	229.2 (219.7-239.2)	
three or more	648	233.4 (220.9-246.7)	
Calculated free estradiol (pmol/L)			
none	356	3.14 (2.92-3.38)	0.320
one	388	2.93 (2.73-3.14)	(0.154)
two	1056	2.90 (2.78-3.02)	
three or more	638	2.92 (2.76-3.08)	
Estrone (pmol/L)			
none	259	209 (197-221)	0.504
one	206	205 (192-219)	(0.375)
two	594	206 (198-214)	
three or more	382	216 (206-226)	
Luteal phase progesterone (nmol/L)			
none	306	26.66 (23.68-30.01)	0.192
one	330	29.70 (26.51-33.28)	(0.653)
two	860	26.34 (24.56-28.26)	
three or more	493	29.01 (26.42-31.85)	
Androstenedione (nmol/L)			
none	408	4.18 (3.99-4.39)	0.582
one	285	4.38 (4.14-4.63)	(0.995)
two	667	4.28 (4.13-4.44)	
three or more	372	4.20 (4.00-4.41)	
DHEAS (nmol/L)			
none	539	2887 (2749-3031)	0.866
one	377	2851 (2693-3019)	(0.934)
two	945	2827 (2726-2931)	
three or more	597	2889 (2760-3025)	
Testosterone (nmol/L)			
none	613	1.00 (0.96-1.05)	0.198
one	511	0.98 (0.94-1.03)	(0.073)
two	1276	0.95 (0.92-0.98)	
three or more	784	0.96 (0.92-0.99)	
Calculated free testosterone (pmol/L)			
none	608	12.8 (12.2-13.5)	0.054
one	511	12.4 (11.7-13.1)	(0.010)
two	1268	11.9 (11.5-12.3)	
three or more	775	11.8 (11.3-12.4)	
SHBG (nmol/L)			
none	619	53.9 (52.0-55.9)	0.284
one	516	54.6 (52.5-56.7)	(0.053)
two	1299	55.4 (54.0-56.8)	
three or more	791	56.5 (54.7-58.3)	



* means are geometric means

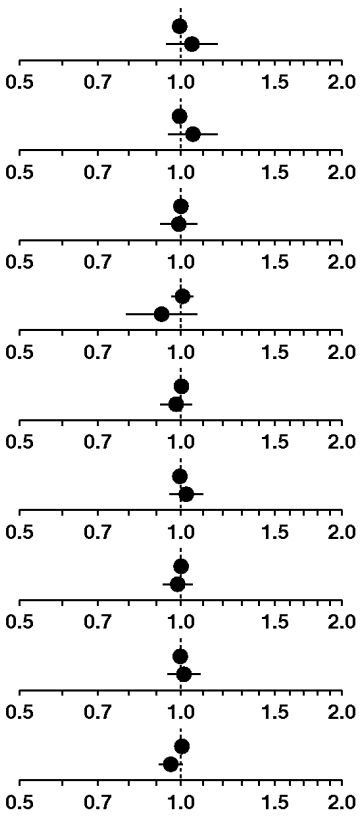
+ means are scaled to the overall mean concentration (dotted line)

Web Figure 24

plotmhpreyTK(11): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of mother or sister with breast cancer, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het	Relative mean+ & 95% CI
Estradiol (pmol/L)				
no	1770	231.5 (223.9-239.3)	0.365	
yes	164	244.0 (218.8-272.1)		
Calculated free estradiol (pmol/L)				
no	1745	2.93 (2.83-3.02)	0.310	
yes	162	3.10 (2.79-3.45)		
Estrone (pmol/L)				
no	820	209 (204-215)	0.833	
yes	92	207 (191-225)		
Luteal phase progesterone (nmol/L)				
no	1575	27.79 (26.52-29.13)	0.269	
yes	152	25.40 (21.81-29.58)		
Androstenedione (nmol/L)				
no	1060	4.27 (4.16-4.38)	0.545	
yes	169	4.17 (3.90-4.47)		
DHEAS (nmol/L)				
no	1723	2849 (2774-2926)	0.487	
yes	241	2928 (2724-3147)		
Testosterone (nmol/L)				
no	2420	0.97 (0.95-0.99)	0.668	
yes	296	0.95 (0.90-1.01)		
Calculated free testosterone (pmol/L)				
no	2400	12.1 (11.8-12.4)	0.677	
yes	294	12.3 (11.5-13.2)		
SHBG (nmol/L)				
no	2420	55.5 (54.6-56.5)	0.077	
yes	297	52.9 (50.3-55.6)		



* means are geometric means

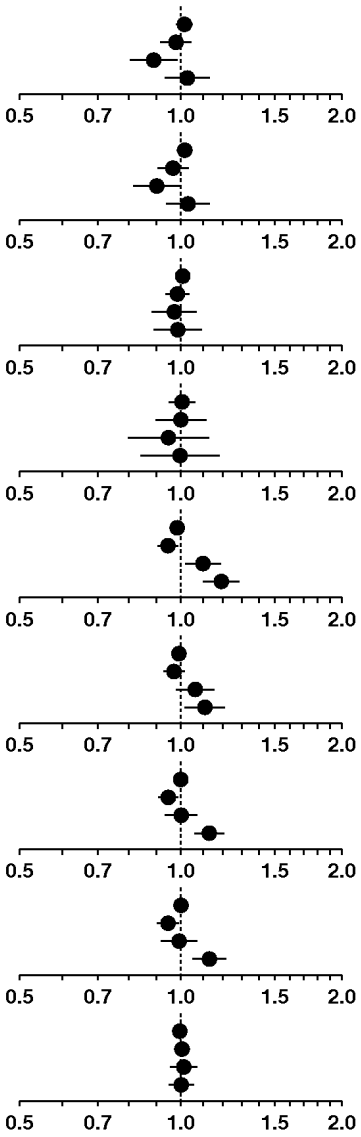
+ means are scaled to the overall mean concentration (dotted line)

Web Figure 25

plotmhpreyTK(19): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of cigarette smoking, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het
Estradiol (pmol/L)			
never	1555	236.3 (228.1-244.8)	0.089
previous	437	227.9 (213.1-243.6)	
current (<15 cigs per day)	192	207.0 (187.0-229.1)	
current (15+ cigs per day)	217	239.2 (217.2-263.4)	
Calculated free estradiol (pmol/L)			
never	1538	2.99 (2.89-3.10)	0.092
previous	429	2.85 (2.66-3.04)	
current (<15 cigs per day)	190	2.65 (2.40-2.93)	
current (15+ cigs per day)	217	3.04 (2.76-3.34)	
Estrone (pmol/L)			
never	974	211 (205-217)	0.808
previous	333	206 (196-217)	
current (<15 cigs per day)	95	203 (185-224)	
current (15+ cigs per day)	84	206 (186-229)	
Luteal phase progesterone (nmol/L)			
never	1293	27.74 (26.18-29.38)	0.943@
previous	362	27.60 (24.74-30.79)	
current (<15 cigs per day)	143	26.16 (21.96-31.15)	
current (15+ cigs per day)	153	27.50 (23.21-32.59)	
Androstenedione (nmol/L)			
never	1012	4.19 (4.07-4.32)	<0.001
previous	449	4.03 (3.86-4.21)	
current (<15 cigs per day)	152	4.68 (4.34-5.05)	
current (15+ cigs per day)	145	5.07 (4.69-5.48)	
DHEAS (nmol/L)			
never	1537	2837 (2758-2919)	0.023
previous	593	2777 (2654-2906)	
current (<15 cigs per day)	180	3044 (2801-3308)	
current (15+ cigs per day)	168	3173 (2911-3458)	
Testosterone (nmol/L)			
never	1931	0.97 (0.94-0.99)	<0.001
previous	651	0.92 (0.88-0.95)	
current (<15 cigs per day)	231	0.97 (0.90-1.04)	
current (15+ cigs per day)	276	1.09 (1.02-1.16)	
Calculated free testosterone (pmol/L)			
never	1918	12.1 (11.8-12.5)	0.001
previous	645	11.5 (10.9-12.0)	
current (<15 cigs per day)	229	12.0 (11.1-13.0)	
current (15+ cigs per day)	276	13.7 (12.8-14.7)	
SHBG (nmol/L)			
never	1965	55.0 (53.9-56.1)	0.930
previous	660	55.5 (53.6-57.5)	
current (<15 cigs per day)	233	56.0 (52.8-59.4)	
current (15+ cigs per day)	284	55.3 (52.5-58.4)	



* means are geometric means

+ means are scaled to the overall mean concentration (dotted line)

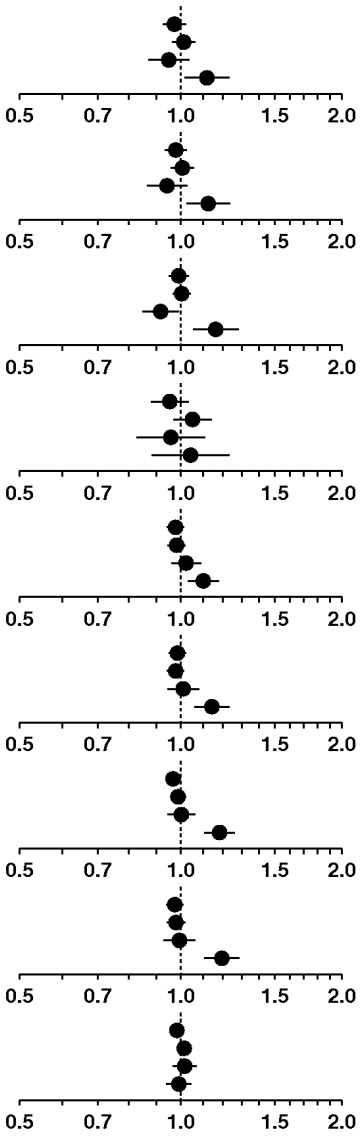
@ indicates significant interaction with study (P<0.05)

Web Figure 26

plotmhpreyTK(13): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of usual alcohol consumption, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het (trend)
Estradiol (pmol/L)			
none	782	226.3 (215.5-237.5)	0.040
<10 g/d	747	235.6 (224.0-247.8)	(0.062)
10-19 g/d	240	220.8 (202.1-241.2)	
20+ g/d	212	260.3 (236.5-286.5)	
Calculated free estradiol (pmol/L)			
none	775	2.88 (2.75-3.02)	0.029
<10 g/d	738	2.96 (2.82-3.12)	(0.078)
10-19 g/d	239	2.77 (2.55-3.02)	
20+ g/d	212	3.31 (3.02-3.64)	
Estrone (pmol/L)			
none	529	207 (199-216)	0.003
<10 g/d	624	210 (202-218)	(0.183)
10-19 g/d	147	192 (177-208)	
20+ g/d	98	243 (220-268)	
Luteal phase progesterone (nmol/L)			
none	679	26.31 (24.30-28.49)	0.334
<10 g/d	660	29.01 (26.72-31.50)	(0.340)
10-19 g/d	204	26.43 (22.83-30.59)	
20+ g/d	163	28.78 (24.33-34.04)	
Androstenedione (nmol/L)			
none	659	4.16 (4.01-4.32)	0.013
<10 g/d	617	4.18 (4.03-4.34)	(0.003)
10-19 g/d	217	4.36 (4.09-4.64)	
20+ g/d	207	4.69 (4.39-5.01)	
DHEAS (nmol/L)			
none	930	2819 (2718-2923)	0.003
<10 g/d	919	2798 (2696-2903)	(0.005)
10-19 g/d	265	2889 (2699-3094)	
20+ g/d	224	3270 (3031-3528)	
Testosterone (nmol/L)			
none	1107	0.93 (0.90-0.96)	<0.001
<10 g/d	999	0.95 (0.92-0.99)	(<0.001)
10-19 g/d	315	0.97 (0.91-1.03)	
20+ g/d	282	1.14 (1.07-1.22)	
Calculated free testosterone (pmol/L)			
none	1103	11.8 (11.4-12.3)	<0.001
<10 g/d	993	11.9 (11.4-12.3)	(<0.001)
10-19 g/d	315	12.1 (11.3-12.9)	
20+ g/d	281	14.5 (13.4-15.6)	
SHBG (nmol/L)			
none	1110	54.3 (52.9-55.8)	0.399
<10 g/d	1005	56.0 (54.5-57.7)	(0.395)
10-19 g/d	317	56.2 (53.4-59.1)	
20+ g/d	287	54.8 (51.9-57.8)	



* means are geometric means

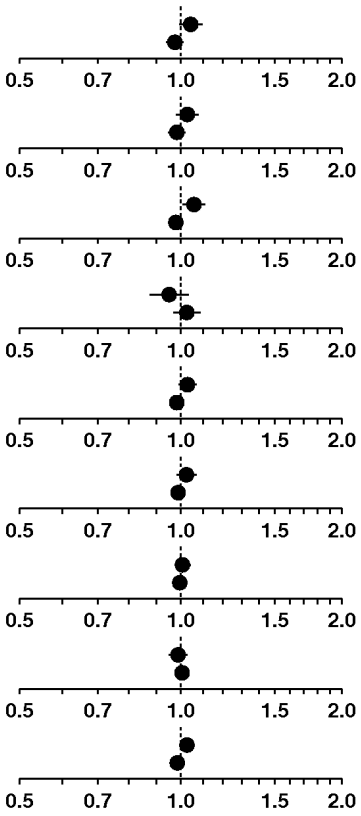
+ means are scaled to the overall mean concentration (dotted line)

Web Figure 27

plotmhpreyTK(14): 10/01/2013

Mean concentration of selected hormones among premenopausal controls by categories of ever used hormonal contraceptives, adjusted for study, age, BMI and phase of cycle

Hormone and category	n	Mean* (95% CI)	P-het
Estradiol (pmol/L)			
no	878	242.8 (231.2-254.9)	0.035
yes	1515	226.8 (218.7-235.2)	
Calculated free estradiol (pmol/L)			
no	873	3.03 (2.88-3.18)	0.158
yes	1492	2.89 (2.79-3.00)	
Estrone (pmol/L)			
no	400	221 (211-232)	0.008
yes	1091	205 (199-211)	
Luteal phase progesterone (nmol/L)			
no	695	26.23 (24.14-28.51)	0.160
yes	1321	28.31 (26.70-30.01)	
Androstenedione (nmol/L)			
no	649	4.38 (4.22-4.55)	0.065
yes	1121	4.18 (4.07-4.30)	
DHEAS (nmol/L)			
no	768	2931 (2812-3056)	0.167
yes	1727	2827 (2752-2905)	
Testosterone (nmol/L)			
no	1068	0.97 (0.94-1.01)	0.597@
yes	2025	0.96 (0.94-0.99)	
Calculated free testosterone (pmol/L)			
no	1062	12.0 (11.5-12.5)	0.492
yes	2009	12.2 (11.9-12.5)	
SHBG (nmol/L)			
no	1107	56.7 (55.2-58.3)	0.021
yes	2034	54.4 (53.3-55.5)	



* means are geometric means
+ means are scaled to the overall mean concentration (dotted line)
@ indicates significant interaction with study (P<0.05)