Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies

Endogenous Hormones and Breast Cancer Collaborative Group[*,1,2]

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BACKGROUND: Breast cancer risk for postmenopausal women is positively associated with circulating concentrations of oestrogens and androgens, but the determinants of these hormones are not well understood.

METHODS: Cross-sectional analyses of breast cancer risk factors and circulating hormone concentrations in more than 6000 postmenopausal women controls in 13 prospective studies.

RESULTS: Concentrations of all hormones were lower in older than younger women, with the largest difference for dehydroepiandrosterone sulphate (DHEAS), whereas sex hormone-binding globulin (SHBG) was higher in the older women. Androgens were lower in women with bilateral ovariectomy than in naturally postmenopausal women, with the largest difference for free testosterone. All hormones were higher in obese than lean women, with the largest difference for free oestradiol, whereas SHBG was lower in obese women. Smokers of 15 + cigarettes per day had higher levels of all hormones than non-smokers, with the largest difference for testosterone. Drinkers of 20 + g alcohol per day had higher levels of all hormones, but lower SHBG, than non-drinkers, with the largest difference for DHEAS. Hormone concentrations were not strongly related to age at menarche, parity, age at first full-term pregnancy or family history of breast cancer.

CONCLUSION: Sex hormone concentrations were strongly associated with several established or suspected risk factors for breast cancer, and may mediate the effects of these factors on breast cancer risk.

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Breast cancer risk for postmenopausal women is positively associated with the circulating concentrations of oestrogens and androgens (Endogenous Hormones and Breast Cancer Collaborative Group, 2002; Missmer *et al*, 2004; Kaaks *et al*, 2005). The increase in risk of postmenopausal breast cancer associated with obesity may be largely explained by the relatively high circulating concentrations of free oestradiol in obese postmenopausal women (Endogenous Hormones and Breast Cancer Collaborative Group, 2003a; Rinaldi *et al*, 2006), but it is unclear to what extent the effects of other risk factors may be mediated by their associations with circulating sex hormones. A number of previous studies have examined the associations of circulating sex hormones with known and possible breast cancer risk factors, such as age at menarche, parity, age at first full-term pregnancy, type of menopause, smoking and alcohol, but they have not been large enough to provide definitive results (see Discussion). The Endogenous Hormones and Breast Cancer Collaborative Group was established to conduct collaborative analyses of individual data from prospective studies of endogenous hormones and breast cancer. We report here the cross-sectional associations of circulating concentrations of sex hormones and sex hormone-binding globulin (SHBG) with 12 demographic, reproductive and lifestyle factors.

MATERIALS AND METHODS

Data collection

Published studies were eligible for the collaborative reanalysis if they included data on endogenous hormones and breast cancer risk using prospectively collected blood samples from postmenopausal women, as described previously (Endogenous Hormones and Breast Cancer Collaborative Group, 2002). Studies were identified by computer-aided literature searches, within relevant review articles, and through our discussions with colleagues. Studies were eligible for inclusion if they had published peer-reviewed data on endogenous hormone concentrations and breast cancer risk using prospectively collected blood samples from postmenopausal women. The data reported here are for the standardised database as available for analysis by the collaborative group in late 2010. The studies included were: CLUE I, Washington County, MD, USA (Wysowski *et al*, 1987; Gordon *et al*, 1990; Helzlsouer *et al*, 1994); Columbia, MO, USA (Dorgan *et al*, 1996, 1997); European Prospective Investigation into Cancer and Nutrition (EPIC), Europe (Kaaks *et al*, 2005); Guernsey, UK (Thomas *et al*, 1997); Malmö/Umeå, Sweden (Manjer *et al*, 2003);

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Table I Assay methods and median hormone and SHBG concentrations

Study, country	Oestradiol			Oestrone			Androstenedione			DHEAS			Testosterone			SHBG		
	Method	CV, %	Median (dl), $pmol\,l^{-1}$	Method	CV, %	Median (dl), $pmol\,l^{-1}$	Method	CV, %	Median (dl), $nmol\,l^{-1}$	Method	CV, %	Median (dl), $nmol\,l^{-1}$	Method	CV, %	Median (dl), $nmol\,l^{-1}$	Method	CV, %	Median (dl), $nmol\,l^{-1}$
CLUE I, USA phase 1	CP RIA	—	161.5	CP RIA	—	183	CP RIA		5.48				CP RIA		0.81			
CLUE I, USA phase 2	E RIA	7.1	58.7	E RIA	9.9	141	E RIA	10.0	2.74	NE RIA	2.4	1815				IA	10.9	55.8
Columbia, MO, USA	NE RIA	23.7	51.4	NE RIA	5.0	129	NE RIA	1.3	3.11	NE RIA	0.7	2204	NE RIA	5.8	0.59	IRMA	1.6	53.4
EPIC, Europe	NE RIA	5.8	89.1	NE RIA	10.2	145	NE RIA	4.8	3.04	NE RIA	7.0	2034	NE RIA	10.8	1.21	IRMA	8.0	34.5
Guernsey, UK	E RIA	15.1	35.0 (3.0)							NE RIA	9	2041	NE RIA	4.5	0.91 (0.35)	IRMA	4.1	63.0
Malmö/Umeå, Sweden				NE RIA	20.6	74 (4.44)	E RIA	⩽15	2.33 (0.14)	NE RIA	⩽15	2730 (200)	E RIA	⩽15	1.22 (0.1)	IMF	⩽15	55.4
MCCS, Australia	NE ECIA	10	57.0 (18)				NE RIA	15	2.15 (0.02)	NE CIA	10	1500 (200)	NE ECIA	7	0.70 (0.1)	IRMA	7	50.7(2)
Nurses' Health Study, USA	E RIA	11.0	22.0 (7.3)	E RIA	12.1	89 (37.0)	E RIA	12.1	1.99 (0.10)	NE RIA	9.0	2313 (136)	E RIA	12.3	0.76 (0.035)	IA	15.5	47.7
NYU WHS, USA	NE RIA	13.2	81.8	NE RIA	14.4	99	NE RIA	13.5	2.46	NE RIA	14.7	2144	NE RIA	15.8	0.79	IRMA	13.5	48.1
ORDET, Italy	E RIA	9.6	21.7							NE RIA	3.7	2004	NE RIA	7.3	1.17	IRMA	4.4	41.5
Rancho Bernardo, USA	E RIA	7.1	40.4 (11)	E RIA	7.7	113 (11)	E RIA	4.3	1.92 (0.06)	NE RIA	10	1755 (52)	E RIA	4.9	0.75 (0.07)	Binding assay	11.4	29.0
RERF, Japan	NE RIA	9.8	65.2							NE RIA	—	952				RIA	5.3	67.3
SOF, USA	E RIA	11	22.0 (7.3)	E RIA	17	74 (37.0)	E RIA	16	1.29 (0.1)	NE RIA	12	1742 (170)	CP RIA	13	0.62 (0.03)	RIA	7.8	42.3 (5)
WHI-OS, USA	NE IA	5.9	40.4 (18.4)															

Abbreviations: CIA = competitive immunoassay; CLUE = Washington County, MD Study 'Give us a clue to cancer and heart disease'; CP = chromatographic purification; CV = coefficient of variation: total if reported, otherwise between assay, otherwise within assay; dl = detection limit; E = extraction step; ECIA = electrochemiluminescence immunoassay; EPIC = European Prospective Investigation into Cancer and Nutrition; IA = immunoassay; IMF = immunofluorometry; IRMA = immunoradiometric assay; MCCS = Melbourne Collaborative Cohort Study; NE = no extraction step; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors; RIA = radioimmunoassay; RERF = Radiation Effects Research Foundation; SHBG = sex hormone-binding globulin; SOF = Study of Osteoporotic Fractures; WHI-OS = Women's Health Initiative, Observational Study.

the Melbourne Collaborative Cohort Study (MCCS), Australia (Baglietto et al, 2009); Nurses' Health Study, USA (Hankinson et al, 1998); New York University Women's Health Study (NYU WHS), USA (Toniolo et al, 1995; Zeleniuch-Jacquotte et al, 1997, 2004); Study of Hormones and Diet in the Etiology of Breast Tumors (ORDET), Italy (Berrino et al, 1996); Rancho Bernardo, USA (Barrett-Connor et al, 1990; Garland et al, 1992); Radiation Effects Research Foundation (RERF), Japan (Kabuto et al, 2000); Study of Osteoporotic Fractures (SOF), USA (Cauley et al, 1999); and the Women's Health Initiative, Observational Study (WHI-OS), USA (Gunter et al, 2009). Details of the recruitment of participants, informed consent and definitions of reproductive variables are in the original publications. Height and weight were measured in eight studies (EPIC (most centres), Guernsey, Malmö/Umeå, MCCS, ORDET, Rancho Bernardo, SOF and WHI-OS) and were self-reported in four studies (Columbia, Nurses' Health Study, NYU WHS and RERF). With the exception of the RERF Study in Japan, the majority of the women in these studies were of white European ethnic origin.

Collaborators were asked to provide data on concentrations of the hormones oestradiol (total), free oestradiol, oestrone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), testosterone, free testosterone and SHBG, where available. Details of the assays and median hormone concentrations for the data included in our analyses are in Table 1. Data from CLUE I were analysed as two substudies because of changes in the assay method for oestradiol, oestrone and androstenedione between the first phase and the second phase. Data for oestradiol from the Malmö/Umeå Study were not included because over 50% of recorded values were below the detection limit of the assay. The majority of studies measured hormone concentrations in serum, whereas some (Malmö/Umeå, MCCS, Nurses' Health Study and Rancho Bernardo) used plasma. Circulating concentrations of free oestradiol and free testosterone were calculated from the concentrations of oestradiol and testosterone, respectively, and SHBG, with albumin assumed to be constant ($40\,g\,l^{-1}$), according to the law of mass action (Sodergard et al, 1982; Endogenous Hormones and Breast Cancer Collaborative Group, 2003b); in the subset of studies that had measured free oestradiol and free testosterone, the correlations of these values with the calculated values were 0.98 and 0.77,

respectively, and using calculated values for all participants increased the sample size for these analyses, while standardizing the estimation approach.

Collaborators also provided data on reproductive and anthropometric factors for each woman in their study. Women who were using hormone replacement therapy or other exogenous sex hormones at the time of blood collection were excluded. All studies that contributed data to the analysis were cohorts in which blood samples were collected from women who were then followed to identify those subjects who developed breast cancer. The analyses in this paper use data only from the 'control' women who had not developed breast cancer during the follow-up period in each contributing study.

Statistical analysis

Hormone and SHBG concentrations were logarithmically transformed to normalise the distributions. A few hormone and SHBG values recorded as zero were reset to a suitably small non-zero value to allow logarithmic transformation: $1\,pmol\,l^{-1}$, 0.01, 0.01 and $1\,nmol\,l^{-1}$ for oestradiol (1 value), androstenedione (3 values), testosterone (16 values) and SHBG (2 values), respectively.

Partial correlations between the hormones and SHBG were computed using study-specific standardised values: $(x_{jk} - m_j)/s_j$, where m_j and s_j denote the mean and standard deviation of the log-transformed hormone concentrations in study j and x_{jk} is an observation from that study. These standardised values were adjusted for age at blood collection (continuous), type of menopause (natural, hysterectomy, bilateral ovariectomy) and body mass index (BMI; continuous) for women with known values for these variables. The use of standardisation effectively adjusts for the differences in mean hormone concentration between studies.

Geometric mean hormone and SHBG concentrations by categories of various factors, together with their 95% confidence intervals, were calculated using the predicted values from analysis of variance models scaled to the overall geometric mean concentration. The factors examined were age (<55, $55-59$, $60-64$, $65-69$ and $70+$ years), type of menopause (natural, hysterectomy, bilateral ovariectomy, other or unknown), BMI

(calculated as weight in kilograms divided by the square of height in metres and categorised as <22.5, $22.5-24.9$, $25.0-27.4$, $27.5-29.9$ and $30.0+$ kg m^{-2}), smoking (never, previous, current <15 cigarettes day and current $15+$ cigarettes per day), alcohol (none, <10 g per day, $10-19$ g per day and $20+$ g per day (a 4 oz glass of wine typically contains 12 g of alcohol)), age at menarche (<12, $12-13$ and $14+$ years), number of full-term pregnancies (none, 1, 2, 3 and $4+$), age at first full-term pregnancy (<25 and $25+$ years), years since menopause ($0-4$, $5-14$ and $15+$ years), family history of breast cancer (no and yes), previous use of hormonal contraceptives (no and yes) and previous use of hormone therapy (no and yes).

The geometric mean hormone concentrations were adjusted for study, age at blood collection, type of menopause and BMI, as appropriate (categories as above, with an extra 'unknown' category for BMI, which was unknown for 4% of women with values for oestradiol). Heterogeneity between categories was assessed by F tests arising from the analysis of variance. Trends in hormone concentrations across categories of all quantitative variables except alcohol were tested by scoring the categories from 1 to the maximum number of categories and treating them as continuous variables in the analysis of variance. Alcohol was treated differently because of the highly skewed distribution of intakes, and the alcohol consumption categories were scored 0, 3, 12 and 30, respectively, in the tests for linear trend in accordance with the median daily alcohol intake in grams in those categories. Values for BMI, smoking, alcohol consumption and most of the other factors investigated were unknown for some women, and these individuals were excluded from the analyses of hormones in relation to these factors. Tests of heterogeneity between studies in the associations of hormones with other factors were obtained by adding a study-by-factor interaction term to the analysis of variance and using the F-test to assess its significance. For oestradiol (and free oestradiol), oestrone, androstenedione and testosterone (and free testosterone), the results were examined according to whether or not the hormone assay had included a purification step (usually by organic solvent extraction and column chromatography). CLUE I; Nurses' Health Study; Rancho Bernardo; and SOF used purification steps in the assays for all of these analytes, and Columbia, MO; EPIC; NYU WHS; RERF; and WHI used all direct assays, whereas Guernsey and ORDET used extraction for oestradiol and direct assays for testosterone, and Malmö/Umeå used direct assays for oestrone and extraction for androstenedione and testosterone. All statistical tests were two-sided, and statistical significance was taken as $P<0.05$. All analyses were performed using Stata Statistical Software release 10 (Stata Corp., College Station, TX, USA).

RESULTS

Collaborating studies and participant characteristics

A total of 13 studies contributed data, 7 in the United States of America, 1 each in Australia, Italy, Japan, Sweden and the United Kingdom, and the multi-centre European study EPIC (Table 2). In all, 6291 women contributed data to the analyses. Mean age at blood sampling ranged from 58.5 years in the Guernsey Study up to 71.7 years in SOF, with an overall mean of 61.7 years. Body mass index was available for all except one study (CLUE I), with an overall mean of 26.4 kg m^{-2}. Type of menopause was available for all except one study (RERF), with most women in the other studies having had a natural (non-surgical) menopause. Smoking data were available for all studies and the proportion of current smokers ranged from 7.2% in SOF to 26.0% in Malmö/Umeå, with an overall proportion of 13.2%. Alcohol consumption data were available for eight studies and the proportion of current alcohol consumers ranged from 37.8% in NYU WHS to 70.0% in WHI-OS, with an overall proportion of 54.5%.

Correlations between hormones

Concentrations of all the sex hormones were positively correlated with each other (Table 3); partial correlation coefficients ranged between 0.29 and 0.93. Free oestradiol was strongly correlated with total oestradiol ($r=0.93$) and inversely correlated with SHBG ($r=-0.42$). Free testosterone was strongly correlated with total testosterone ($r=0.89$) and inversely correlated with SHBG ($r=-0.41$). Sex hormone-binding globulin was weakly inversely correlated with each of the remaining sex hormones, except for testosterone. Similar correlations between hormones and SHBG were observed for studies that had used assays with a purification

Table 2 Participant characteristics

Study, country	Number of women[a]	Mean age, years	Age range, years	Mean BMI, kg m^{-2}	Natural menopause, %	Current smokers, %	Alcohol consumers, %
CLUE I, USA	194	61.4	38–96	NA	100.0	22.4	NA
Columbia, MO, USA	133	61.8	50–76	26.6	72.2	12.8	NA
EPIC, Europe[b]	1304	60.1	44–76	26.8	82.5	14.7	66.3
Guernsey, UK	177	58.5	45–79	25.6	94.9	18.8	NA
Malmö/Umeå, Sweden	436	60.0	46–73	26.0	96.1	26.0	69.0
MCCS, Australia	1146	60.4	40–70	27.4	79.4	8.5	40.8
Nurses' Health Study, USA	641	61.4	46–69	26.2	74.8	12.5	58.1
NYU WHS, USA	563	58.9	44–65	25.4	76.7	8.1	37.8
ORDET, Italy	245	58.1	47–69	26.6	78.4	10.2	56.3
Rancho Bernardo, USA	582	66.7	50–79	24.6	72.5	22.6	NA
RERF, Japan	56	59.3	41–77	22.1	NA	16.7	NA
SOF, USA	378	71.7	65–87	26.6	86.2	7.2	41.0
WHI-OS, USA	436	64.5	50–80	27.7	65.1	7.4	70.0
All studies	6291	61.7	38–96	26.4	80.1	13.2	54.5

Abbreviations: BMI, body mass index; CLUE = Washington County, MD Study 'Give us a clue to cancer and heart disease'; DHEAS = dehydroepiandrosterone sulphate; EPIC = European Prospective Investigation into Cancer and Nutrition; MCCS = Melbourne Collaborative Cohort Study; NA = not available; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors; RERF = Radiation Effects Research Foundation; SHBG = sex hormone-binding globulin; SOF = Study of Osteoporotic Fractures; WHI-OS = Women's Health Initiative, Observational Study. [a]Number of women with one or more sex hormone measurements. The numbers of women with measurements of oestradiol, free oestradiol, oestrone, androstenedione, DHEAS, testosterone, free testosterone and SHBG were 5614, 5003, 3848, 5181, 5389, 5666, 5494 and 5678, respectively. [b]France, Germany, Greece, Italy, The Netherlands, Spain and the United Kingdom.

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Table 3 Correlations between hormones and SHBG

	Free oestradiol	Oestrone	Androstenedione	DHEAS	Testosterone	Free testosterone	SHBG
Oestradiol	0.93	0.50	0.29	0.29	0.35	0.35	−0.08
Free oestradiol		0.50	0.29	0.31	0.32	0.47	−0.42
Oestrone			0.44	0.45	0.41	0.42	−0.14
Androstenedione				0.60	0.59	0.56	−0.08
DHEAS					0.56	0.56	−0.11
Testosterone						0.89	0.02
Free testosterone							−0.41

Abbreviations: DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin. Partial correlation coefficients between standardized log-transformed hormone and SHBG concentrations, adjusted for age at blood collection, type of menopause and body mass index. The number of pairs of observations for each partial correlation is between 2945 and 5081. All values are statistically significant at $P < 0.0001$, except for the partial correlation between testosterone and SHBG.

step for oestradiol, oestrone, androstenedione and testosterone and for studies that had used direct assays for these hormones (results not shown).

Age

All the hormones and SHBG were significantly associated with age but not always in a linear pattern (Figure 1). Concentrations of oestradiol, oestrone and testosterone were highest in the youngest age group (below 55 years), after which they declined and did not vary much thereafter. Androstenedione and DHEAS were 23% and 44% lower, respectively, in women aged 70 years and above than in women aged below 55 years. Sex hormone-binding globulin was 21% higher in women aged 70 years and above than in women aged below 55 years. Calculated free oestradiol and calculated free testosterone were 16% and 18% lower, respectively, in women aged 70 years and above than in women aged below 55 years. There was significant heterogeneity ($P < 0.01$) between studies in the associations of oestradiol, oestrone, testosterone, free testosterone and SHBG with age; for these hormones (but not for SHBG), there was no significant association with age from studies that had used assays with a purification step, but there were significant inverse associations with age from studies that had used direct assays (results not shown).

Women who had a natural menopause within 5 years of blood collection had significantly higher circulating concentrations of oestradiol and free oestradiol than women who had a natural menopause at least 5 years previously (Table 4). Excluding women who had undergone menopause < 5 years before blood collection, and women aged below 60 years at blood collection who had an unknown age at menopause, reduced but did not eliminate the difference in oestradiol, free oestradiol and oestrone concentrations between women aged < 55 and older women, but had little impact on the associations of age with the other hormones and SHBG (results not shown).

Type of menopause

Oestradiol, oestrone and SHBG did not differ significantly by type of menopause (Figure 2). Calculated free oestradiol was 7% lower in women who had undergone a bilateral ovariectomy than in women who had a natural menopause. Compared with women with a natural menopause, concentrations of androstenedione and DHEAS were 5% and 10% lower, respectively, for women who had had a hysterectomy and 13% and 11% lower, respectively, for women who had had a bilateral ovariectomy. Testosterone was 15% lower for women who had had a hysterectomy and 30% lower for those who had had a bilateral ovariectomy than for women who had had a natural menopause, with similar differences in calculated free testosterone. There was significant heterogeneity between studies in the associations of androstenedione and testosterone with type of menopause; for androstenedione, the association with type of menopause was not significant for studies

that had used a purification step but was significant for those that had used direct assays, whereas for testosterone the association with type of menopause was significant regardless of whether the assays had used a purification step (results not shown).

Body mass index

All the hormones and SHBG were significantly associated with BMI (Figure 3). Oestrogen concentrations were much higher in obese (BMI at least $30 \, \text{kg} \, \text{m}^{-2}$) than in thin women (BMI $< 22.5 \, \text{kg} \, \text{m}^{-2}$): oestradiol by 47%, calculated free oestradiol by 89% and oestrone by 47%. Concentrations of three androgens were also higher in obese than in thin women: androstenedione by 16%, testosterone by 17% and calculated free testosterone by 72%, whereas DHEAS showed significant heterogeneity by BMI but no clear trend. Sex hormone-binding globulin was 46% lower in obese women than in thin women. The associations of oestrogens, androgens and SHBG with BMI were similar in the subgroup of women who had undergone a bilateral ovariectomy (results not shown). There was significant heterogeneity between studies in the associations of oestradiol, free oestradiol and SHBG with BMI; for oestradiol and free oestradiol, the difference between extreme categories of BMI was significant regardless of the type of assay, but was larger in the studies that had used a purification step than in the studies that had used direct assays (increases from the lowest to the highest BMI category of 82% and 30%, respectively, for oestradiol and 129% and 70%, respectively, for free oestradiol; results not shown).

Smoking

Concentrations of all the sex hormones were higher for women who smoked 15 + cigarettes per day than for never smokers (Figure 4). The largest differences were for androstenedione, testosterone and calculated free testosterone, which were 18%, 20% and 19%, respectively, higher for heavy cigarette smokers than for never smokers. Sex hormone-binding globulin did not differ according to smoking status. There was no significant heterogeneity between studies in the associations of smoking with the hormones or SHBG. The associations of hormones with smoking were less marked before adjustment for BMI, but were almost unaltered after further adjustment for alcohol (results not shown).

Alcohol

Concentrations of all the sex hormones were higher for women who consumed 20 + g per day of alcohol than for non-drinkers (Figure 5). The largest difference was for DHEAS, which was 25% higher for women consuming 20 + g per day of alcohol than for non-drinkers. Circulating SHBG was 10% lower for women consuming 20 + g per day of alcohol than for non-drinkers. There was no significant heterogeneity between studies in the associations of alcohol with the hormones or SHBG. The associations of hormones with alcohol were less marked before adjustment for
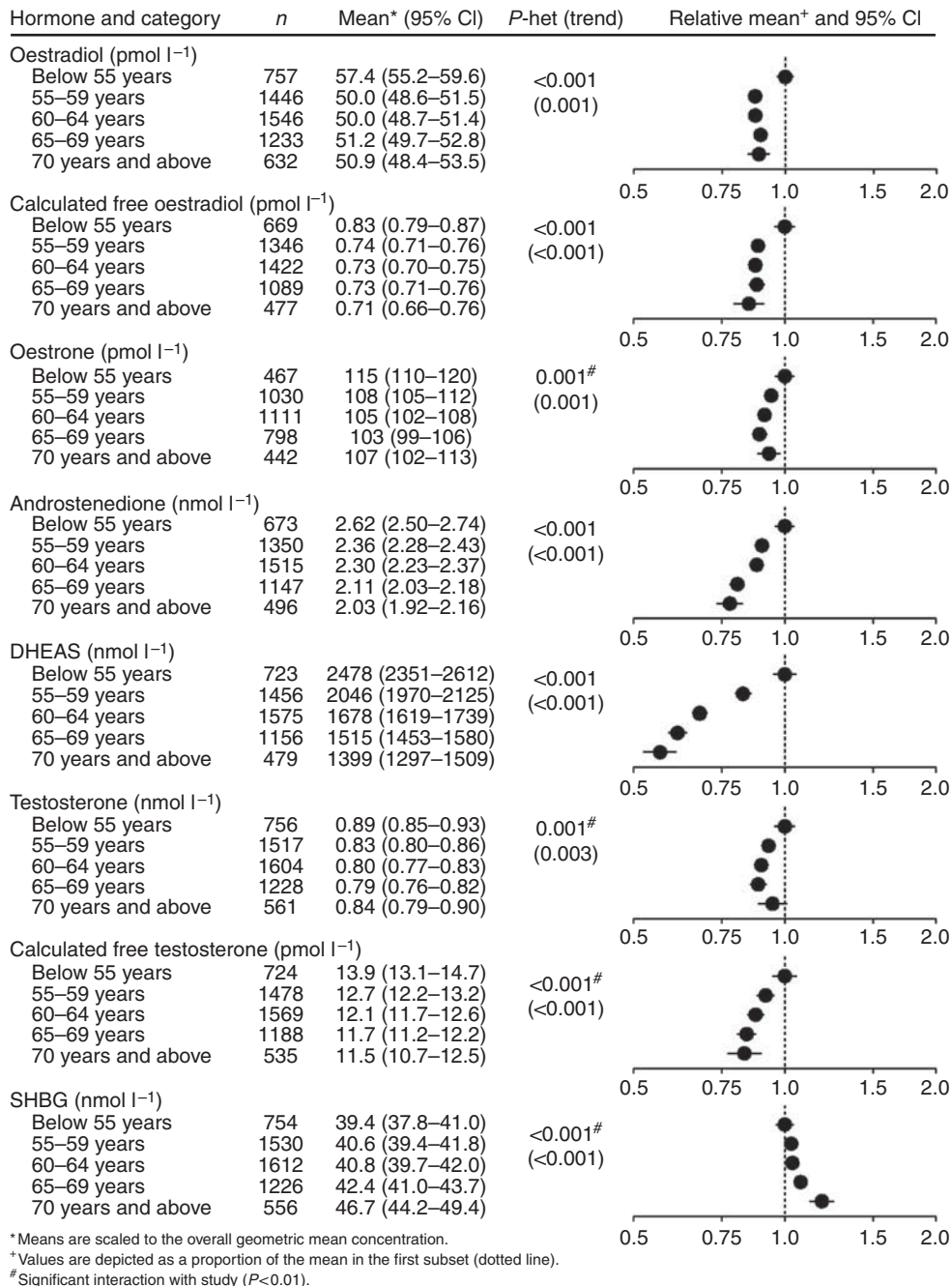
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Hormone and category	n	Mean* (95% CI)	P-het (trend)
Oestradiol (pmol l⁻¹)			
Below 55 years	757	57.4 (55.2–59.6)	<0.001
55–59 years	1446	50.0 (48.6–51.5)	(0.001)
60–64 years	1546	50.0 (48.7–51.4)	
65–69 years	1233	51.2 (49.7–52.8)	
70 years and above	632	50.9 (48.4–53.5)	
Calculated free oestradiol (pmol l⁻¹)			
Below 55 years	669	0.83 (0.79–0.87)	<0.001
55–59 years	1346	0.74 (0.71–0.76)	(<0.001)
60–64 years	1422	0.73 (0.70–0.75)	
65–69 years	1089	0.73 (0.71–0.76)	
70 years and above	477	0.71 (0.66–0.76)	
Oestrone (pmol l⁻¹)			
Below 55 years	467	115 (110–120)	0.001#
55–59 years	1030	108 (105–112)	(0.001)
60–64 years	1111	105 (102–108)	
65–69 years	798	103 (99–106)	
70 years and above	442	107 (102–113)	
Androstenedione (nmol l⁻¹)			
Below 55 years	673	2.62 (2.50–2.74)	<0.001
55–59 years	1350	2.36 (2.28–2.43)	(<0.001)
60–64 years	1515	2.30 (2.23–2.37)	
65–69 years	1147	2.11 (2.03–2.18)	
70 years and above	496	2.03 (1.92–2.16)	
DHEAS (nmol l⁻¹)			
Below 55 years	723	2478 (2351–2612)	<0.001
55–59 years	1456	2046 (1970–2125)	(<0.001)
60–64 years	1575	1678 (1619–1739)	
65–69 years	1156	1515 (1453–1580)	
70 years and above	479	1399 (1297–1509)	
Testosterone (nmol l⁻¹)			
Below 55 years	756	0.89 (0.85–0.93)	0.001#
55–59 years	1517	0.83 (0.80–0.86)	(0.003)
60–64 years	1604	0.80 (0.77–0.83)	
65–69 years	1228	0.79 (0.76–0.82)	
70 years and above	561	0.84 (0.79–0.90)	
Calculated free testosterone (pmol l⁻¹)			
Below 55 years	724	13.9 (13.1–14.7)	<0.001#
55–59 years	1478	12.7 (12.2–13.2)	(<0.001)
60–64 years	1569	12.1 (11.7–12.6)	
65–69 years	1188	11.7 (11.2–12.2)	
70 years and above	535	11.5 (10.7–12.5)	
SHBG (nmol l⁻¹)			
Below 55 years	754	39.4 (37.8–41.0)	<0.001#
55–59 years	1530	40.6 (39.4–41.8)	(<0.001)
60–64 years	1612	40.8 (39.7–42.0)	
65–69 years	1226	42.4 (41.0–43.7)	
70 years and above	556	46.7 (44.2–49.4)	

*Means are scaled to the overall geometric mean concentration.
⁺Values are depicted as a proportion of the mean in the first subset (dotted line).
#Significant interaction with study (P<0.01).

Figure 1 Geometric mean concentration of selected hormones in postmenopausal women by categories of age at blood collection, adjusted for study, type of menopause and BMI.

BMI, but were almost unaltered after further adjustment for smoking (results not shown).

Reproductive and other factors

Oestradiol was 6% lower in women who had menarche at ages 14 years and above than in women who had menarche before 12 years of age (Table 4). Age at menarche was not associated with the other hormones or SHBG.

Parity was not associated with any of the hormones or with SHBG, with the exception of DHEAS, which varied significantly between categories but with no clear pattern (Table 4). For parous women, none of the hormones or SHBG varied significantly by age at first full-term pregnancy.

Time since menopause was associated with oestradiol, calculated free oestradiol and DHEAS, which were 12%, 17% and 8% higher, respectively, in women who had had their menopause within the previous 5 years than in women who had had their menopause 15 or more years previously (Table 4). The other hormones and SHBG were not associated with time since menopause. Examination of time since menopause of 0–4 years in finer categories (0–1, 2, 3 and 4) showed that the highest concentrations of oestradiol were in the category of 0–1 years since menopause (64.4 pmol l⁻¹), with little difference across the other categories (55.7, 57.2 and 53.7 pmol l⁻¹, respectively); a

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Table 4 Relationships of circulating concentrations of sex hormones and SHBG in postmenopausal women with reproductive and other factors. Values are geometric means and 95% confidence intervals adjusted for study, age at blood collection, type of menopause and body mass index and scaled to the overall geometric mean concentration

Factor and subset	Oestradiol (pmol l⁻¹)	Calculated free oestradiol (pmol l⁻¹)	Oestrone (pmol l⁻¹)	Androstenedione (nmol l⁻¹)	DHEAS (nmol l⁻¹)	Testosterone (nmol l⁻¹)	Calculated free T (pmol l⁻¹)	SHBG (nmol l⁻¹)
Age at menarche (years)								
Below 12	53.6 (51.6–55.6)	0.77 (0.74–0.81)	109 (105–114)	2.26 (2.16–2.37)	1816 (1722–1914)	0.82 (0.78–0.86)	12.3 (11.7–13.0)	41.5 (40.1–43.0)
12–13	51.2 (50.2–52.3)	0.74 (0.72–0.75)	105 (102–107)	2.30 (2.24–2.36)	1795 (1743–1848)	0.82 (0.80–0.84)	12.3 (11.9–12.7)	41.9 (41.1–42.7)
14 and above	50.5 (49.3–51.7)	0.74 (0.72–0.76)	109 (106–112)	2.27 (2.20–2.33)	1790 (1733–1849)	0.82 (0.80–0.84)	12.4 (12.0–12.8)	40.8 (39.9–41.7)
P for heterogeneity (trend)	0.033 (0.015)	0.114 (0.108)	0.055 (0.664)	0.708 (0.839)	0.903 (0.689)	0.977 (0.986)	0.883 (0.684)	0.195 (0.187)
Number of full-term pregnancies								
None	51.8 (49.8–53.8)	0.76 (0.72–0.79)	110 (105–115)	2.27 (2.16–2.38)	1702 (1610–1798)	0.82 (0.78–0.86)	12.5 (11.8–13.2)	40.9 (39.4–42.4)
One	53.0 (50.8–55.3)	0.76 (0.73–0.80)	108 (103–112)	2.32 (2.21–2.44)	1797 (1699–1902)	0.84 (0.80–0.89)	12.8 (12.1–13.5)	40.3 (38.8–41.9)
Two	51.6 (50.2–53.0)	0.74 (0.72–0.77)	107 (104–110)	2.32 (2.25–2.39)	1864 (1799–1932)	0.82 (0.79–0.85)	12.3 (11.9–12.8)	41.7 (40.7–42.7)
Three	50.6 (49.1–52.2)	0.74 (0.71–0.76)	106 (103–110)	2.28 (2.20–2.36)	1841 (1766–1921)	0.83 (0.80–0.86)	12.5 (12.0–13.1)	41.4 (40.2–42.6)
Four or more	50.4 (48.9–52.1)	0.73 (0.70–0.75)	104 (100–108)	2.22 (2.13–2.30)	1704 (1627–1784)	0.79 (0.76–0.83)	11.8 (11.3–12.4)	42.1 (40.8–43.4)
P for heterogeneity (trend)	0.361 (0.092)	0.465 (0.083)	0.477 (0.071)	0.490 (0.326)	0.006 (0.956)	0.383 (0.267)	0.221 (0.103)	0.464 (0.143)
Age at first full-term pregnancy[a] (years)								
Below 25	51.8 (50.6–53.0)	0.75 (0.73–0.77)	107 (105–110)	2.28 (2.22–2.35)	1797 (1740–1856)	0.82 (0.80–0.85)	12.4 (12.0–12.8)	41.2 (40.4–42.1)
25 and above	50.8 (49.7–52.0)	0.73 (0.72–0.75)	106 (104–109)	2.28 (2.22–2.34)	1794 (1741–1849)	0.82 (0.80–0.84)	12.3 (11.9–12.7)	41.6 (40.8–42.4)
P for heterogeneity	0.249	0.262	0.646	0.955	0.937	0.865	0.640	0.535
Years since menopause[b]								
0–4	57.3 (54.6–60.0)	0.84 (0.79–0.88)	109 (104–115)	2.30 (2.17–2.43)	1941 (1819–2071)	0.82 (0.77–0.87)	12.4 (11.7–13.3)	40.1 (38.4–41.9)
5–14	49.5 (48.3–50.7)	0.72 (0.70–0.74)	105 (102–108)	2.26 (2.20–2.32)	1750 (1693–1810)	0.81 (0.79–0.84)	12.3 (11.9–12.7)	41.2 (40.3–42.2)
15+	51.1 (49.2–53.0)	0.72 (0.69–0.75)	108 (104–113)	2.31 (2.21–2.41)	1790 (1697–1888)	0.84 (0.80–0.88)	12.4 (11.8–13.1)	42.6 (41.1–44.2)
P for heterogeneity (trend)	<0.001 (0.003)	<0.001 (<0.001)	0.216 (0.922)	0.664 (0.844)	0.017 (0.129)	0.612 (0.603)	0.896 (0.945)	0.194 (0.072)
Family history of breast cancer								
No	51.1 (49.9–52.4)	0.74 (0.72–0.76)	107 (104–109)	2.29 (2.22–2.36)	1805 (1743–1870)	0.81 (0.79–0.84)	12.2 (11.8–12.7)	41.5 (40.6–42.3)
Yes	52.1 (49.4–54.9)	0.76 (0.71–0.81)	107 (102–113)	2.25 (2.10–2.42)	1745 (1608–1894)	0.86 (0.80–0.93)	13.0 (11.9–14.1)	41.2 (39.2–43.3)
P for heterogeneity	0.534	0.366	0.851	0.704	0.459	0.170	0.210	0.826
Previous use of hormonal contraceptives								
No	50.6 (49.6–51.5)	0.73 (0.71–0.74)	106 (104–108)	2.33 (2.28–2.39)	1796 (1751–1843)	0.83 (0.81–0.85)	12.4 (12.0–12.7)	41.7 (41.0–42.4)
Yes	53.0 (51.5–54.5)	0.77 (0.75–0.80)	109 (106–112)	2.18 (2.11–2.25)	1795 (1727–1865)	0.81 (0.78–0.84)	12.3 (11.8–12.8)	40.7 (39.7–41.8)
P for heterogeneity	0.010	0.002	0.164	0.001	0.972	0.283	0.895	0.129
Previous use of hormone therapy[b]								
No	51.2 (50.3–52.1)	0.74 (0.73–0.76)	109 (106–111)	2.31 (2.25–2.36)	1809 (1762–1858)	0.83 (0.81–0.85)	12.5 (12.1–12.8)	41.5 (40.8–42.2)
Yes	51.9 (49.9–54.0)	0.75 (0.72–0.79)	99 (95–104)	2.16 (2.04–2.28)	1731 (1630–1837)	0.77 (0.73–0.82)	11.7 (11.0–12.4)	41.2 (39.6–42.8)
P for heterogeneity	0.542	0.582	0.001	0.027	0.186	0.022	0.050	0.729

Abbreviations: DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin. [a]Parous women only. [b]Natural postmenopausal women only. *P*-values relate to tests of heterogeneity across categories (trend), the latter obtained by scoring the categories 1, 2, 3… as required.

similar pattern was seen for calculated free oestradiol (means of 0.93, 0.81, 0.83 and 0.80 pmol l⁻¹, respectively).

Family history of breast cancer was not associated with any of the hormones or SHBG (Table 4). Women who had previously used hormonal contraceptives had 5% higher oestradiol and calculated free oestradiol and 6% lower androstenedione than women who had never used hormonal contraceptives. Women who had previously used hormonal therapy for menopause had 9% lower oestrone, 6% lower androstenedione and 7% lower testosterone than women who had not used hormonal therapy for menopause.

DISCUSSION

The results suggest that circulating concentrations of sex hormones are associated with several of the well-established or suspected risk factors for breast cancer. The strongest associations were with BMI: concentrations of all the hormones were higher in obese women than in women with a low BMI, with the largest differences for calculated free oestradiol and free testosterone. Ovariectomy was associated with low levels of androgens, and cigarette smoking and alcohol were associated with moderate increases in all the sex hormones. Age at menarche, parity, age at

first full-term pregnancy and family history of breast cancer were not strongly related to any of the hormones examined.

The large sample size provides sufficient power to detect differences in circulating hormone concentrations between categories as small as 10% for most of the analyses presented. Some of the observations replicate well-established associations, whereas others provide novel information of possible relevance to understanding the aetiology of breast cancer, as discussed in more detail below. Many previous publications on this topic are from the studies participating in this collaboration, and these publications are cited together with publications from studies not included in this collaboration. Limitations of this analysis are that the data are cross-sectional, and therefore cannot be used to attribute causality, and that some other potentially important factors such as physical activity and diet were not included in the analyses. Another potential limitation is that, with the exception of the RERF Study, the majority of the women in the contributing studies were of white European ethnic origin.

For some of the analyses, there was significant heterogeneity in the results from different studies. Inspection of the results from individual studies did not show large between-study differences in the variation of hormone levels by categories of risk factors, and suggested that the heterogeneity was largely due to differences in the magnitudes of the associations rather than qualitative
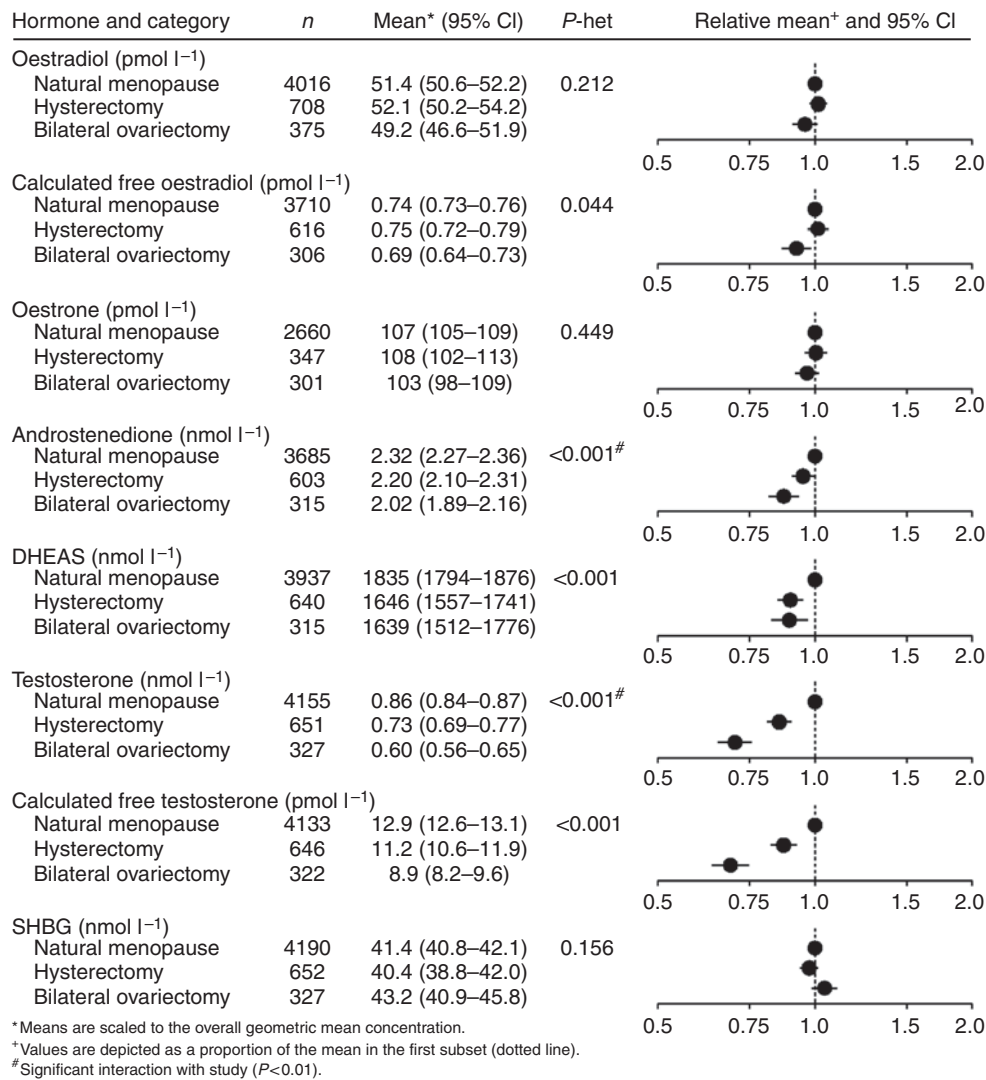
Hormone and category	n	Mean* (95% CI)	P-het	Relative mean+ and 95% CI
Oestradiol (pmol l⁻¹)				
Natural menopause	4016	51.4 (50.6–52.2)	0.212	
Hysterectomy	708	52.1 (50.2–54.2)		
Bilateral ovariectomy	375	49.2 (46.6–51.9)		
Calculated free oestradiol (pmol l⁻¹)				
Natural menopause	3710	0.74 (0.73–0.76)	0.044	
Hysterectomy	616	0.75 (0.72–0.79)		
Bilateral ovariectomy	306	0.69 (0.64–0.73)		
Oestrone (pmol l⁻¹)				
Natural menopause	2660	107 (105–109)	0.449	
Hysterectomy	347	108 (102–113)		
Bilateral ovariectomy	301	103 (98–109)		
Androstenedione (nmol l⁻¹)				
Natural menopause	3685	2.32 (2.27–2.36)	<0.001#	
Hysterectomy	603	2.20 (2.10–2.31)		
Bilateral ovariectomy	315	2.02 (1.89–2.16)		
DHEAS (nmol l⁻¹)				
Natural menopause	3937	1835 (1794–1876)	<0.001	
Hysterectomy	640	1646 (1557–1741)		
Bilateral ovariectomy	315	1639 (1512–1776)		
Testosterone (nmol l⁻¹)				
Natural menopause	4155	0.86 (0.84–0.87)	<0.001#	
Hysterectomy	651	0.73 (0.69–0.77)		
Bilateral ovariectomy	327	0.60 (0.56–0.65)		
Calculated free testosterone (pmol l⁻¹)				
Natural menopause	4133	12.9 (12.6–13.1)	<0.001	
Hysterectomy	646	11.2 (10.6–11.9)		
Bilateral ovariectomy	322	8.9 (8.2–9.6)		
SHBG (nmol l⁻¹)				
Natural menopause	4190	41.4 (40.8–42.1)	0.156	
Hysterectomy	652	40.4 (38.8–42.0)		
Bilateral ovariectomy	327	43.2 (40.9–45.8)		

*Means are scaled to the overall geometric mean concentration.
+Values are depicted as a proportion of the mean in the first subset (dotted line).
#Significant interaction with study (P<0.01).

Figure 2 Geometric mean concentration of selected hormones in postmenopausal women by categories of type of menopause, adjusted for study, age at blood collection and BMI.

differences. It is likely that the accuracy of the assays used varied; therefore, the results should be interpreted simply as an average across assays for the available data. The results suggested that the type of sex hormone assay (with or without a purification step) influenced some of the results. For example, the association of oestradiol with BMI was stronger in the studies that had used an assay with previous purification (see below); in general, hormone assays that include a purification step are both more sensitive and more specific than direct, non-extraction assays (Stanczyk et al, 2003).

For oestradiol and testosterone, we calculated estimates of the concentration of the free hormone using the law of mass action and the concentration of SHBG, and assumed a fixed concentration of albumin. We chose not to report estimates of non-SHBG-bound oestradiol and testosterone, which are often described as 'bioavailable' because these hormones dissociate readily from albumin. It should be noted that the calculated non-SHBG-bound oestradiol and testosterone would be perfectly correlated with free oestradiol and testosterone, respectively, and therefore that the associations reported for free oestradiol and free testosterone would be identical for the non-SHBG-bound concentrations of these hormones.

The hormones were all positively correlated with each other, presumably because they are all part of the same metabolic pathway. Whereas oestrogen and progesterone production in premenopausal women is controlled by feedback mechanisms through the anterior pituitary and the hypothalamus, the control of sex hormone production in postmenopausal women is not well understood. The oestrogens are derived from the androgens, which are secreted by the adrenal glands and by the stromal tissue of the ovaries. Adrenal androgen production may be stimulated by stress, as ACTH stimulates the adrenal cortex to synthesise cortisol and, less specifically, the adrenal androgens (Azziz et al, 1990; McKenna et al, 1997). Conversion of androgens to oestrogens is catalysed by the enzyme aromatase, and the major site of aromatase activity in postmenopausal women is in the adipose tissue (Grodin et al, 1973).

Age

The large decrease in DHEAS and androstenedione, the small changes in oestrogens and testosterone and the increase in SHBG with age are broadly consistent with previous reports (Meldrum et al, 1981; Moore et al, 1987; Verkasalo et al, 2001; Spencer et al, 2007). The smaller decreases in oestrogens than in two of the three androgens might suggest that the amount of substrate available only partly determines oestrogen concentrations, and that synthesis by aromatisation may become more active in older women (Misso et al, 2005).
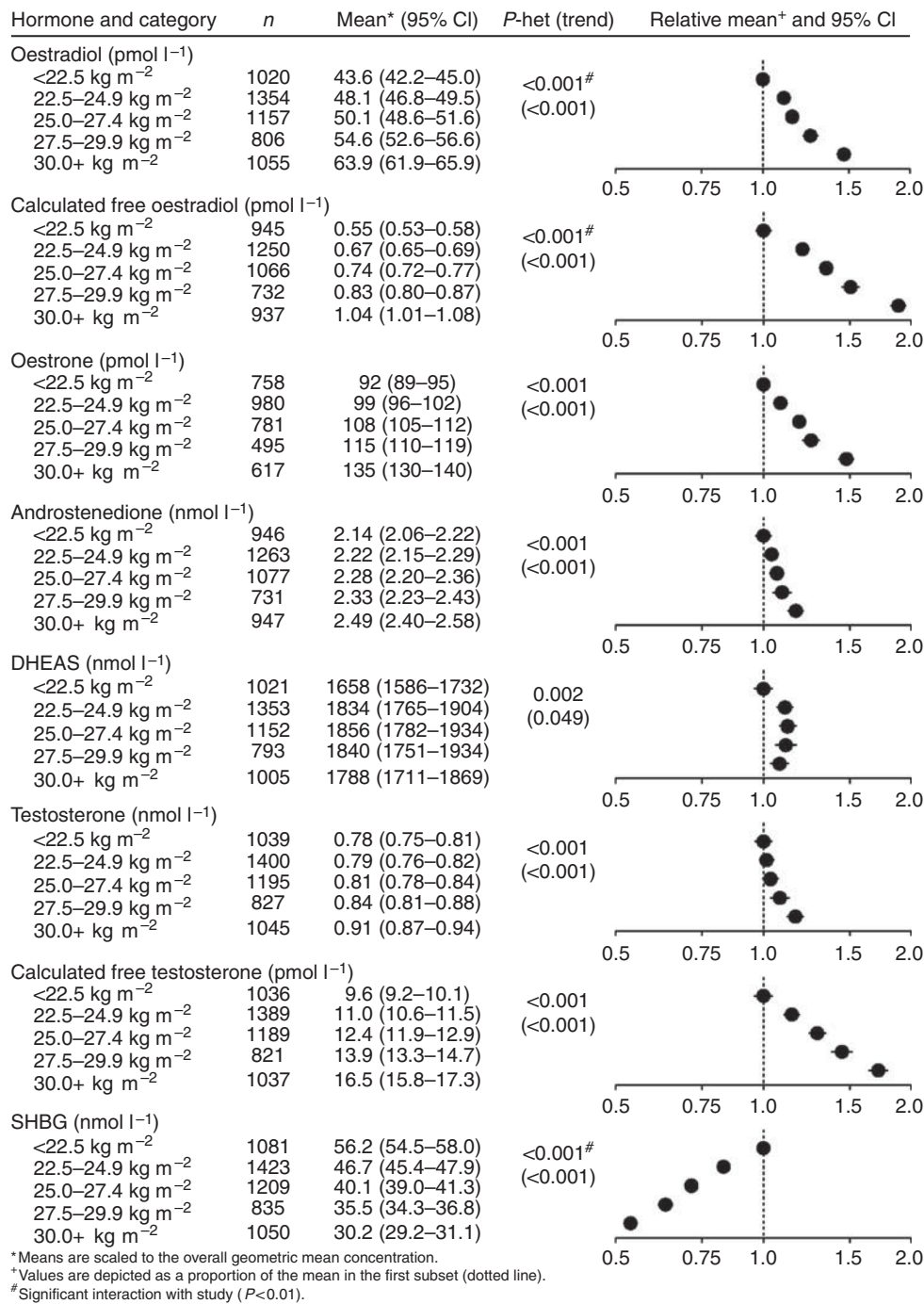
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Hormone and category	n	Mean* (95% CI)	P-het (trend)	Relative mean+ and 95% CI
Oestradiol (pmol l⁻¹)			<0.001# (<0.001)	
<22.5 kg m⁻²	1020	43.6 (42.2–45.0)		
22.5–24.9 kg m⁻²	1354	48.1 (46.8–49.5)		
25.0–27.4 kg m⁻²	1157	50.1 (48.6–51.6)		
27.5–29.9 kg m⁻²	806	54.6 (52.6–56.6)		
30.0+ kg m⁻²	1055	63.9 (61.9–65.9)		
Calculated free oestradiol (pmol l⁻¹)			<0.001# (<0.001)	
<22.5 kg m⁻²	945	0.55 (0.53–0.58)		
22.5–24.9 kg m⁻²	1250	0.67 (0.65–0.69)		
25.0–27.4 kg m⁻²	1066	0.74 (0.72–0.77)		
27.5–29.9 kg m⁻²	732	0.83 (0.80–0.87)		
30.0+ kg m⁻²	937	1.04 (1.01–1.08)		
Oestrone (pmol l⁻¹)			<0.001 (<0.001)	
<22.5 kg m⁻²	758	92 (89–95)		
22.5–24.9 kg m⁻²	980	99 (96–102)		
25.0–27.4 kg m⁻²	781	108 (105–112)		
27.5–29.9 kg m⁻²	495	115 (110–119)		
30.0+ kg m⁻²	617	135 (130–140)		
Androstenedione (nmol l⁻¹)			<0.001 (<0.001)	
<22.5 kg m⁻²	946	2.14 (2.06–2.22)		
22.5–24.9 kg m⁻²	1263	2.22 (2.15–2.29)		
25.0–27.4 kg m⁻²	1077	2.28 (2.20–2.36)		
27.5–29.9 kg m⁻²	731	2.33 (2.23–2.43)		
30.0+ kg m⁻²	947	2.49 (2.40–2.58)		
DHEAS (nmol l⁻¹)			0.002 (0.049)	
<22.5 kg m⁻²	1021	1658 (1586–1732)		
22.5–24.9 kg m⁻²	1353	1834 (1765–1904)		
25.0–27.4 kg m⁻²	1152	1856 (1782–1934)		
27.5–29.9 kg m⁻²	793	1840 (1751–1934)		
30.0+ kg m⁻²	1005	1788 (1711–1869)		
Testosterone (nmol l⁻¹)			<0.001 (<0.001)	
<22.5 kg m⁻²	1039	0.78 (0.75–0.81)		
22.5–24.9 kg m⁻²	1400	0.79 (0.76–0.82)		
25.0–27.4 kg m⁻²	1195	0.81 (0.78–0.84)		
27.5–29.9 kg m⁻²	827	0.84 (0.81–0.88)		
30.0+ kg m⁻²	1045	0.91 (0.87–0.94)		
Calculated free testosterone (pmol l⁻¹)			<0.001 (<0.001)	
<22.5 kg m⁻²	1036	9.6 (9.2–10.1)		
22.5–24.9 kg m⁻²	1389	11.0 (10.6–11.5)		
25.0–27.4 kg m⁻²	1189	12.4 (11.9–12.9)		
27.5–29.9 kg m⁻²	821	13.9 (13.3–14.7)		
30.0+ kg m⁻²	1037	16.5 (15.8–17.3)		
SHBG (nmol l⁻¹)			<0.001# (<0.001)	
<22.5 kg m⁻²	1081	56.2 (54.5–58.0)		
22.5–24.9 kg m⁻²	1423	46.7 (45.4–47.9)		
25.0–27.4 kg m⁻²	1209	40.1 (39.0–41.3)		
27.5–29.9 kg m⁻²	835	35.5 (34.3–36.8)		
30.0+ kg m⁻²	1050	30.2 (29.2–31.1)		

*Means are scaled to the overall geometric mean concentration.
+Values are depicted as a proportion of the mean in the first subset (dotted line).
#Significant interaction with study (P<0.01).

Figure 3 Geometric mean concentration of selected hormones in postmenopausal women by categories of BMI, adjusted for study, age at blood collection and type of menopause.

It is likely that the relatively high concentrations of oestradiol in women aged below 55 years are associated with the fact that many of these women have had their menopause within the last 5 years, and exclusion of women who had recently had their menopause reduced this association (see further discussion below in relation to time since menopause).

Hysterectomy and ovariectomy

Previous analyses of data from studies contributing to this collaboration (Laughlin et al, 2000) and others (Judd et al, 1974a; Vermeulen, 1976; Davison et al, 2005; Cappola et al, 2007; Fogle et al, 2007; McTiernan et al, 2008) have shown that postmenopausal women who have had a bilateral ovariectomy have lower circulating androgen concentrations than postmenopausal women with intact ovaries. Furthermore, clinical studies have shown that androgen concentrations are higher in blood samples from the ovarian veins than in the peripheral circulation, demonstrating that the ovaries secrete androgens (Grodin et al, 1973; Judd et al, 1974b). Our analyses replicated these relationships; women who had undergone a bilateral ovariectomy had testosterone concentrations about 30% lower than those of
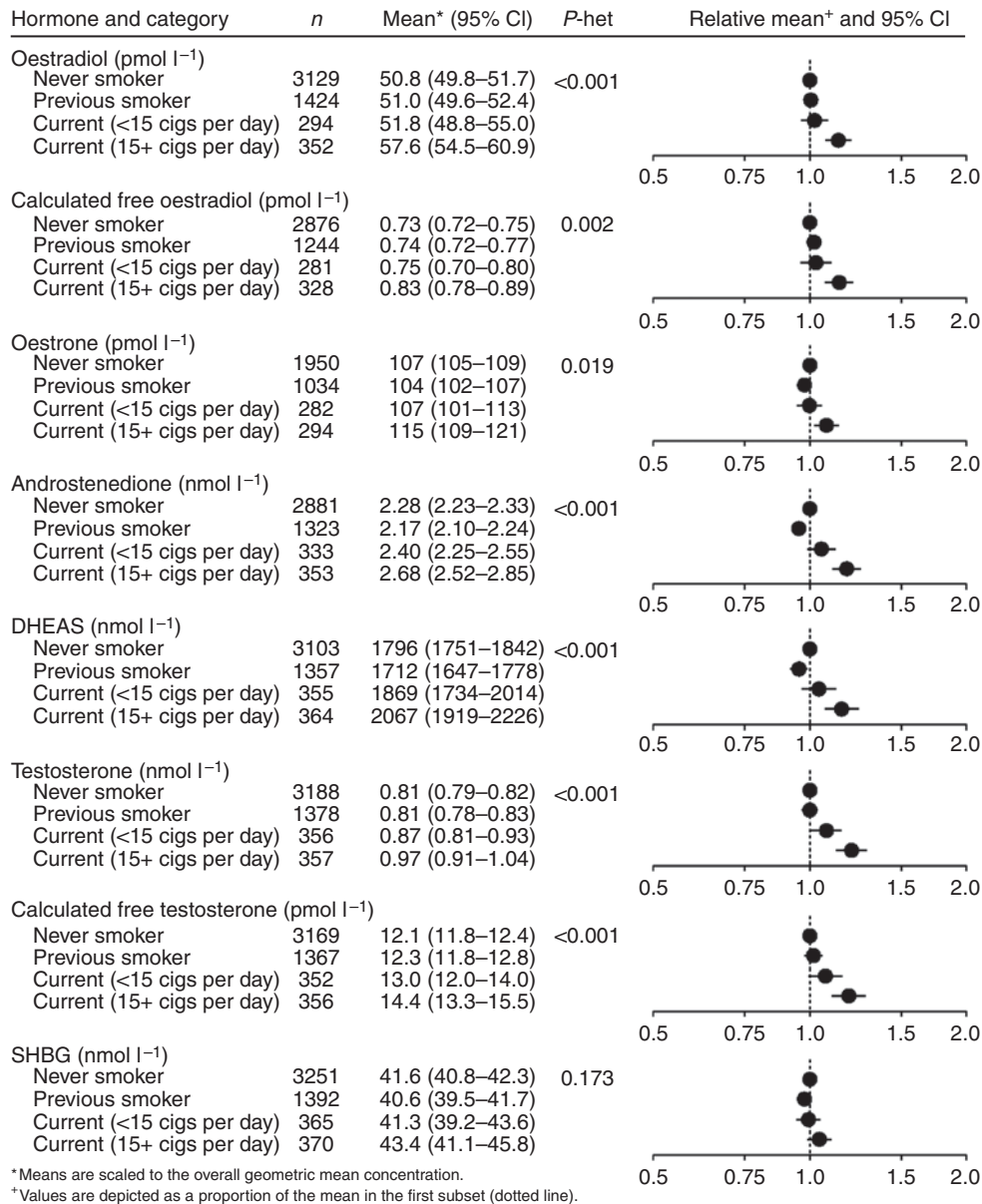
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Hormone and category	n	Mean* (95% CI)	P-het
Oestradiol (pmol l^{-1})			
Never smoker	3129	50.8 (49.8–51.7)	<0.001
Previous smoker	1424	51.0 (49.6–52.4)	
Current (<15 cigs per day)	294	51.8 (48.8–55.0)	
Current (15+ cigs per day)	352	57.6 (54.5–60.9)	
Calculated free oestradiol (pmol l^{-1})			
Never smoker	2876	0.73 (0.72–0.75)	0.002
Previous smoker	1244	0.74 (0.72–0.77)	
Current (<15 cigs per day)	281	0.75 (0.70–0.80)	
Current (15+ cigs per day)	328	0.83 (0.78–0.89)	
Oestrone (pmol l^{-1})			
Never smoker	1950	107 (105–109)	0.019
Previous smoker	1034	104 (102–107)	
Current (<15 cigs per day)	282	107 (101–113)	
Current (15+ cigs per day)	294	115 (109–121)	
Androstenedione (nmol l^{-1})			
Never smoker	2881	2.28 (2.23–2.33)	<0.001
Previous smoker	1323	2.17 (2.10–2.24)	
Current (<15 cigs per day)	333	2.40 (2.25–2.55)	
Current (15+ cigs per day)	353	2.68 (2.52–2.85)	
DHEAS (nmol l^{-1})			
Never smoker	3103	1796 (1751–1842)	<0.001
Previous smoker	1357	1712 (1647–1778)	
Current (<15 cigs per day)	355	1869 (1734–2014)	
Current (15+ cigs per day)	364	2067 (1919–2226)	
Testosterone (nmol l^{-1})			
Never smoker	3188	0.81 (0.79–0.82)	<0.001
Previous smoker	1378	0.81 (0.78–0.83)	
Current (<15 cigs per day)	356	0.87 (0.81–0.93)	
Current (15+ cigs per day)	357	0.97 (0.91–1.04)	
Calculated free testosterone (pmol l^{-1})			
Never smoker	3169	12.1 (11.8–12.4)	<0.001
Previous smoker	1367	12.3 (11.8–12.8)	
Current (<15 cigs per day)	352	13.0 (12.0–14.0)	
Current (15+ cigs per day)	356	14.4 (13.3–15.5)	
SHBG (nmol l^{-1})			
Never smoker	3251	41.6 (40.8–42.3)	0.173
Previous smoker	1392	40.6 (39.5–41.7)	
Current (<15 cigs per day)	365	41.3 (39.2–43.6)	
Current (15+ cigs per day)	370	43.4 (41.1–45.8)	

*Means are scaled to the overall geometric mean concentration.
+Values are depicted as a proportion of the mean in the first subset (dotted line).

Figure 4 Geometric mean concentration of selected hormones in postmenopausal women by categories of smoking, adjusted for study, age at blood collection, type of menopause and BMI.

naturally postmenopausal women, together with smaller reductions in androstenedione and DHEAS, but no significant differences for total oestradiol or oestrone. Women who had undergone a hysterectomy without bilateral ovariectomy had circulating androgen concentrations intermediate between those of women who had a natural menopause and women who had undergone a bilateral ovariectomy; this intermediate effect might be due to misclassification of some women who had undergone bilateral ovariectomy into the hysterectomy category and/or damage to the ovarian blood supply during hysterectomy (Laughlin et al, 2000); alternatively, it is possible that long-standing differences in circulating androgens somehow influence the likelihood of hysterectomy.

Body mass index

Levels of the oestrogens were higher in women with higher BMI, probably because women with more adipose tissue have more aromatase (in their adipose tissue) and therefore increased total aromatase activity (Grodin et al, 1973), and the high oestrogen levels of obese postmenopausal women probably explain their increased risk for breast cancer (Endogenous Hormones and Breast Cancer Collaborative Group, 2003a). This relationship has been observed in many previous studies that are not included in this collaboration (Meldrum et al, 1981; Kaye et al, 1991; Madigan et al, 1998; Nagata et al, 2000; Verkasalo et al, 2001; Boyapati et al, 2004; McTiernan et al, 2006; Chavez-MacGregor et al, 2008). Androstenedione and testosterone also increased with increasing BMI, whereas DHEAS was not clearly related to BMI. These associations have been observed previously (Cappola et al, 2007), but the reason why androgens increase with BMI is not clear. Obesity is associated with increases in insulin, which may stimulate androgen production in the ovarian stroma (Barber et al, 2006), whereas adrenal androgen synthesis is not known to be stimulated by insulin; however, our results suggested that androgens also increased with BMI in women who had undergone
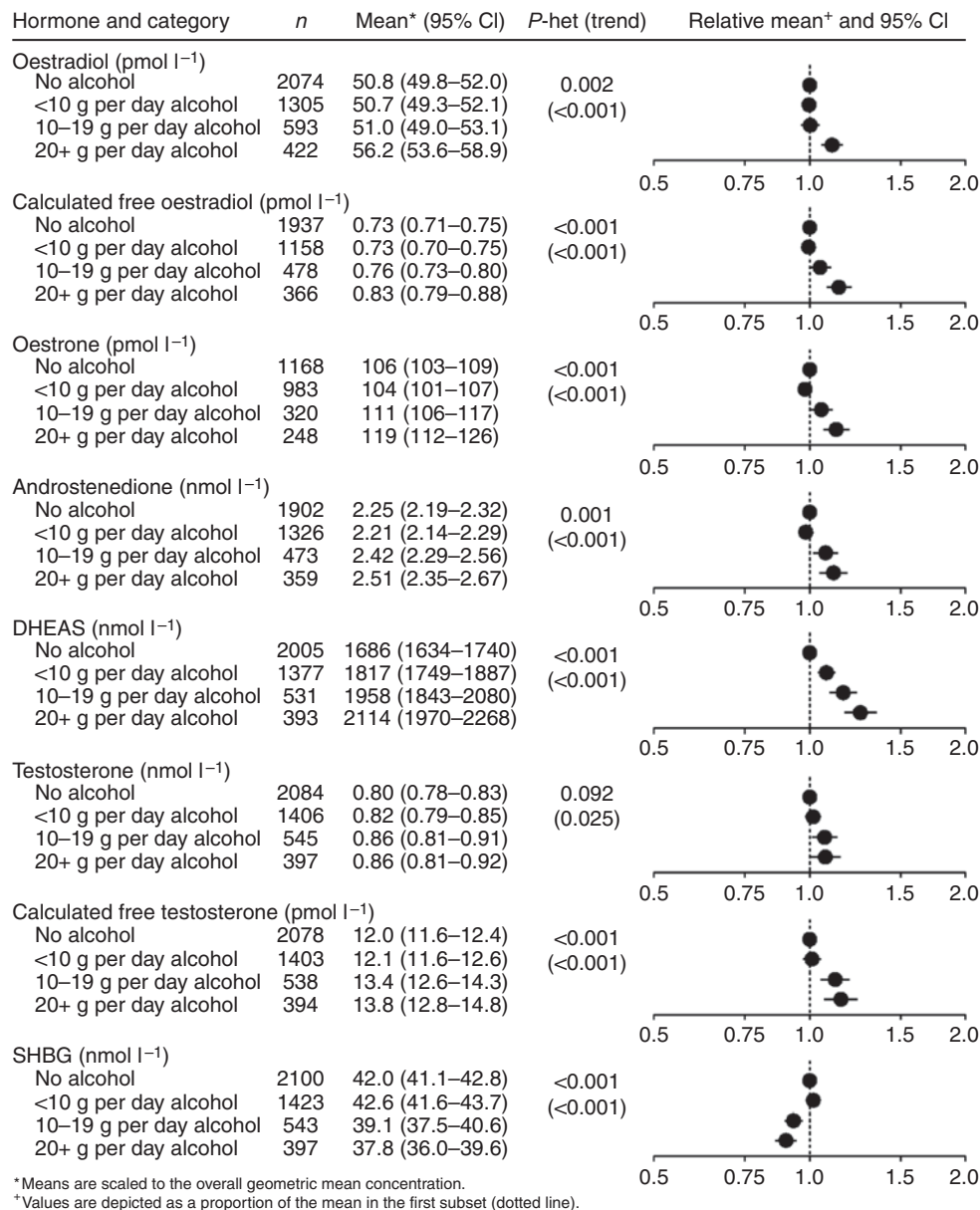
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Hormone and category	n	Mean* (95% CI)	P-het (trend)	Relative mean+ and 95% CI
Oestradiol (pmol l⁻¹)				
No alcohol	2074	50.8 (49.8–52.0)	0.002	
<10 g per day alcohol	1305	50.7 (49.3–52.1)	(<0.001)	
10–19 g per day alcohol	593	51.0 (49.0–53.1)		
20+ g per day alcohol	422	56.2 (53.6–58.9)		
Calculated free oestradiol (pmol l⁻¹)				
No alcohol	1937	0.73 (0.71–0.75)	<0.001	
<10 g per day alcohol	1158	0.73 (0.70–0.75)	(<0.001)	
10–19 g per day alcohol	478	0.76 (0.73–0.80)		
20+ g per day alcohol	366	0.83 (0.79–0.88)		
Oestrone (pmol l⁻¹)				
No alcohol	1168	106 (103–109)	<0.001	
<10 g per day alcohol	983	104 (101–107)	(<0.001)	
10–19 g per day alcohol	320	111 (106–117)		
20+ g per day alcohol	248	119 (112–126)		
Androstenedione (nmol l⁻¹)				
No alcohol	1902	2.25 (2.19–2.32)	0.001	
<10 g per day alcohol	1326	2.21 (2.14–2.29)	(<0.001)	
10–19 g per day alcohol	473	2.42 (2.29–2.56)		
20+ g per day alcohol	359	2.51 (2.35–2.67)		
DHEAS (nmol l⁻¹)				
No alcohol	2005	1686 (1634–1740)	<0.001	
<10 g per day alcohol	1377	1817 (1749–1887)	(<0.001)	
10–19 g per day alcohol	531	1958 (1843–2080)		
20+ g per day alcohol	393	2114 (1970–2268)		
Testosterone (nmol l⁻¹)				
No alcohol	2084	0.80 (0.78–0.83)	0.092	
<10 g per day alcohol	1406	0.82 (0.79–0.85)	(0.025)	
10–19 g per day alcohol	545	0.86 (0.81–0.91)		
20+ g per day alcohol	397	0.86 (0.81–0.92)		
Calculated free testosterone (pmol l⁻¹)				
No alcohol	2078	12.0 (11.6–12.4)	<0.001	
<10 g per day alcohol	1403	12.1 (11.6–12.6)	(<0.001)	
10–19 g per day alcohol	538	13.4 (12.6–14.3)		
20+ g per day alcohol	394	13.8 (12.8–14.8)		
SHBG (nmol l⁻¹)				
No alcohol	2100	42.0 (41.1–42.8)	<0.001	
<10 g per day alcohol	1423	42.6 (41.6–43.7)	(<0.001)	
10–19 g per day alcohol	543	39.1 (37.5–40.6)		
20+ g per day alcohol	397	37.8 (36.0–39.6)		

*Means are scaled to the overall geometric mean concentration.
+Values are depicted as a proportion of the mean in the first subset (dotted line).

Figure 5 Geometric mean concentration of selected hormones in postmenopausal women by categories of usual alcohol consumption, adjusted for study, age at blood collection, type of menopause and BMI.

a bilateral ovariectomy and in whom most androgen synthesis must be in the adrenals. Sex hormone-binding globulin also decreased with increasing BMI, as has been consistently observed in many studies (e.g., Baglietto *et al*, 2009; Moore *et al*, 1987; and Newcomb *et al*, 1995); this decrease may be due to raised insulin concentrations inhibiting SHBG synthesis in the liver (Crave *et al*, 1995).

Overall, total oestradiol was 47% higher and calculated free oestradiol was 89% higher in obese women than in thin women. There was significant heterogeneity between studies in these estimates, and some of this heterogeneity was explained by the oestradiol assay method, with increases of total oestradiol between thin and obese women of 82% for studies that used assays with previous purification but only 30% for studies that used direct non-extraction assays. A similar difference has been noted previously, and it is likely that the larger estimate of 82% is more

accurate because assays with previous purification for oestradiol are more sensitive and more specific than direct assays (Lee *et al*, 2006).

Smoking

We observed higher concentrations of oestrogens and androgens in current heavy cigarette smokers (at least 15 cigarettes per day) than in non-smokers, whereas SHBG did not vary significantly according to smoking status. Cigarette smokers were on average thinner than non-smokers, and the differences in oestrogens were accentuated by the adjustment for BMI. Previous studies have not shown clear associations of smoking with circulating oestrogens, but have generally observed that at least some of the androgens were higher in smokers than in non-smokers (Khaw *et al*, 1988; Cassidenti *et al*, 1992; Baron *et al*, 1995; Manjer *et al*, 2005). The

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mechanism for these associations is unknown but may involve more general effects on the hypothalamic–pituitary–adrenal axis (Kapoor and Jones, 2005).

Most epidemiological studies have suggested that smoking has little or no effect on breast cancer risk (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; International Agency for Research on Cancer, 2004), but some recent large cohort studies have suggested that a long duration of smoking does have a small positive association with breast cancer risk in postmenopausal women, particularly in association with starting to smoke at a young age (Cui et al, 2006; Luo et al, 2011) and perhaps because of the chemical carcinogens in tobacco smoke (Secretan et al, 2009). The moderate differences between heavy smokers and non-smokers in circulating hormones might affect breast cancer risk, although the total effect of smoking on lifetime exposure to hormones also includes the relatively early menopause of smokers (Midgette and Baron, 1990) and possibly changes in oestrogen metabolism (Michnovicz et al, 1986).

Alcohol

Alcohol intake was positively associated with all the sex hormones, with the strongest association for DHEAS, whereas SHBG was inversely associated with alcohol consumption.

High alcohol consumers were on average thinner than non-drinkers, and the differences in oestrogens and in SHBG were accentuated by the adjustment for BMI. These findings are broadly consistent with those of previous observational studies (Onland-Moret et al, 2005). Furthermore, a randomised trial in 51 postmenopausal women showed that DHEAS increased by 5.1% in women consuming 15 g of alcohol per day and by 7.5% in women consuming 30 g of alcohol per day; alcohol consumption also increased circulating concentrations of oestrone sulphate (Dorgan et al, 2001). In another randomised crossover trial in nine postmenopausal women, 30 g of alcohol per day increased circulating DHEAS by 18% but had no detectable effect on oestradiol or testosterone (Sierksma et al, 2004). The mechanism for these associations is unknown.

Alcohol is mostly consumed in the evening, whereas blood samples are usually collected during the day. As the half-life of oestradiol is ∼3 h (Ginsburg et al, 1998), it is possible that there is a marked increase of oestradiol shortly after alcohol consumption in the evening, which cannot be assessed in typical epidemiological studies. In a study of the acute effects of 40 g of alcohol in premenopausal women, circulating oestradiol concentrations increased and reached a peak 25 min after alcohol ingestion (Mendelson et al, 1988). The half-life of DHEAS is longer (∼14 h), and this might explain why this sulphated hormone has the strongest association with alcohol consumption (Dorgan et al, 2001).

The increases in sex hormone concentrations associated with alcohol consumption might contribute to the increase in breast cancer risk with alcohol consumption (Baan et al, 2007), although other factors not related to circulating hormone concentrations might mediate the effect (Seitz and Stickel, 2007).

Other risk factors

Early research suggested that young age at menarche is positively associated with oestrogen levels in young women (MacMahon et al, 1982), but previous studies have not demonstrated clear associations of age at menarche with circulating hormones in postmenopausal women (Verkasalo et al, 2001). We found that, after adjusting for BMI, mean concentrations of oestradiol were 6% lower in postmenopausal women who had had a late menarche than in those who had an early menarche, but age at menarche was not clearly associated with the other hormones or SHBG.

The mean concentration of DHEAS varied significantly with parity, but without a clear pattern. Previous studies have reported that parity was not associated with sex hormones in postmenopausal women (Ness et al, 2000) and that DHEAS was not significantly related to parity in postmenopausal women (Key et al, 1990). Age at first full-term pregnancy in parous women was not associated with any of the sex hormones or SHBG.

Age at menarche, parity and age at first full-term pregnancy are risk factors for breast cancer (Kelsey et al, 1993). Apart from the very weak association of age at menarche with circulating oestradiol, the current findings that these risk factors are not associated with circulating sex hormone concentrations in postmenopausal women suggest that their effects on breast cancer risk are not likely to be mediated through postmenopausal hormone levels. These risk factors may operate through long-term effects on sex hormone levels in premenopausal women (MacMahon et al, 1982), through changes in the duration rather than the level of exposure to premenopausal sex hormones (Key and Pike, 1988), by hormonally mediated long-lasting changes in breast structure (Russo et al, 2005) or by other mechanisms.

Mean concentrations of oestradiol, free oestradiol and DHEAS were significantly higher in women who had had their menopause within 5 years of blood collection than in other women, but the other sex hormones and SHBG did not differ significantly between these groups. The higher levels of oestradiol and free oestradiol were observed primarily up to about 1 year after menopause, and this is compatible with results from a longitudinal study in which oestradiol levels declined from about 2 years before the final menstrual period until ∼2 years after (Sowers et al, 2008). In another report from the same longitudinal study (Crawford et al, 2009), there was a small increase in circulating concentrations of DHEAS during the menopausal transition, followed by a decline (more than 2 years since last menstrual period).

Previous use of exogenous sex hormones was significantly associated with several endogenous hormones, although the magnitude of these associations was small. Previous use of oral contraceptives was associated with higher concentrations of oestradiol and free oestradiol and lower concentrations of androstenedione; previous use of hormonal therapy for menopause was associated with lower concentrations of oestrone, androstenedione, testosterone and free testosterone. In another study (Chan et al, 2008), previous use of oral contraceptives was associated with lower concentrations of oestradiol, oestrone, androstenedione, testosterone and SHBG, and previous use of hormonal therapy for the menopause was associated with lower concentrations of testosterone and 17α-hydroxyprogesterone. It is possible that the associations with hormonal therapy for menopause may be partly because women with relatively low endogenous hormone levels may have more symptoms, and therefore be more likely to be prescribed hormonal therapy.

CONCLUSIONS

Circulating sex hormone concentrations in postmenopausal women are strongly associated with several established or suspected risk factors for breast cancer, and may mediate the effects of these factors on breast cancer risk.

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Conflict of interest

The authors declare no conflict of interest.

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