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Anthropometric measures, endogenous sex steroids and breast cancer risk in postmenopausal women: A study within the EPIC cohort

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In a large case–control study on breast cancer risk and serum hormone concentrations, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, we examined to what extent the relationship of excess body weight with breast cancer risk may be explained by changes in sex steroids. Height, weight, waist and hip circumferences, and serum measurements of testosterone [T], androstenedione [Δ_4], dehydroepiandrosterone sulphate [DHEAS], estradiol [E_2], estrone [E_1] and sex-hormone binding globulin [SHBG] were available for 613 breast cancer cases, and 1,139 matched controls, who were all menopausal at the time of blood donation. Free T [fT] and free E_2 [fE_2] were calculated using mass action equations. Breast cancer risk was related to body mass index (BMI) (RR = 1.11 [0.99–1.25], per 5 kg/m^2 increase in BMI), and waist (RR = 1.12 [1.02–1.24], per 10 cm increase) and hip circumferences (RR = 1.14 [1.02–1.27], per 10 cm increase). The increase in breast cancer risk associated with adiposity was substantially reduced after adjustment for any estrogens, especially for fE_2 (from 1.11 [0.99–1.25] to 0.99 [0.87–1.12], from 1.12 [1.02–1.24] to 1.02 [0.92–1.14] and from 1.14 [1.02–1.27] to 1.05 [0.93–1.18] for BMI, waist and hip circumferences, respectively). A modest attenuation in excess risk was observed after adjustment for fT, but the remaining androgens had little effect on the association of body adiposity with breast cancer. Our data indicate that the relationship of adiposity with breast cancer in postmenopausal women could be partially explained by the increases in endogenous estrogens, and by a decrease in levels of SHBG.

There is abundant evidence that overweight and obesity increase the risk of postmenopausal breast cancer.[1–6] Metaanalyses have shown an ~3% increase in risk per unit increase (kg/m^2) in body mass index (BMI).[7] Apart from BMI, weight, waist circumference or ratio of waist and hip circumferences (WHR), generally, have also been associated with an increased breast cancer risk,[6,8–12] even though most of the time the association between

Abbreviations: Δ_4, Androstenedione; BMI, body mass index; CI, confidence interval; DHEAS, dehydroepiandrosterone sulphate; E_2, estradiol; E_1, estrone; EPIC, European prospective investigation into cancer and nutrition; IRMA, immunoradiometric assays; OR, odds ratio; RIA, radioimmunoassays; SHBG, sex-hormone binding globulin; T, testosterone.

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WHR and waist circumference with breast cancer risk had been shown to disappear after adjustment for overall obesity (BMI).[6,13]

One of the possible mechanisms through which excess adiposity is thought to favor breast tumor development is a change in endogenous sex hormone metabolism.[3] After menopause, the adipose tissue becomes the main site of estrogen synthesis, through the aromatization of the androgens produced by the ovaries or adrenal glands.[14] Increased adiposity, therefore, tends to be associated with higher circulating levels of estrogens.[15,16] In addition, excess weight—and especially increased abdominal fat—causes insulin resistance and chronically elevated blood insulin levels, which in turn lower the hepatic synthesis and blood levels of sex hormone binding globulin (SHBG).[17–20] Lower levels of SHBG result in an increase of the bioavailable fraction of both testosterone and estradiol.

We recently published results from a full cohort analysis of breast cancer risk in relation to anthropometric indices of excess weight among female participants in the European Prospective Investigation into Cancer and Nutrition (EPIC)—a multicenter study aimed at investigating the relationships between lifestyle factors, metabolism, genetic predisposition and cancer risk.[6] In this previous analysis, weight, BMI and measurements of hip circumference were all found to be positively associated with breast cancer risk in postmenopausal women who did not use hormone replacement therapy. A parallel, large nested case–control study within the EPIC cohort (677 breast cancer cases and 1,309 control subjects) showed an increased risk of breast cancer among postmenopausal women who had elevated prediagnostic serum concentrations of estrogens (estradiol [E_2], free estradiol [fE_2], estrone [E_1]) and androgens (testosterone [T], free testosterone [fT], androstenedione ([Δ_4] and dehydroepiandrosterone sulphate [DHEAS]), and who had comparatively low serum levels of SHBG.[21] In the present study, we examine to what extent the relationship of anthropometric measurements (BMI, weight, waist, hip and WHR) with breast cancer risk in postmenopausal women in the EPIC cohort may be explained by the changes in serum concentrations of total and bioavailable estrogens (E_2, fE_2, E_1), or androgens (T, fT, Δ_4 and DHEAS).

Material and methods

Study subjects

The EPIC cohort consists of about 370,000 women and 150,000 men, recruited between 1992 and 1998 in 10 western European countries.[22] All subjects provided extensive standardized questionnaire data on diet and nondietary variables, as well as anthropometric measurements. In all countries included in the present analysis, except for part of the cohort recruited through the Oxford research center, height, weight, and waist and hip circumferences were measured according to standardized protocols, in light clothing. In part of the Oxford cohort, height, weight and body circumferences were self-reported. All measurements were reported to the nearest centimeter (height, body circumferences), and to the nearest kilogram (weight). About 240,000 women and 140,000 men also provided a blood sample. The present study includes breast cancer cases and control subjects from 7 of the EPIC countries: the Netherlands, the United Kingdom, Germany, Spain, Italy, Greece and France. Norway was not included because only very few cases of breast cancer have cumulated after blood collection so far, since blood samples have been collected only recently on a subsample of cohort participants, and Sweden and Denmark were not included because independent studies on breast cancer risk and endogenous sex hormones have been, or are being, conducted separately.

A detailed description of the EPIC cohort (participants, questionnaires data and anthropometry, collection and storage of blood samples, determination of menopausal status at blood donation, and follow-up for cancer incidence and vital status) has been given elsewhere.[21]

Selection of cases and controls

Case and control subjects were selected among women who were postmenopausal at the time of blood donation (postmenopausal status defined as not having had any menses over the past 12 months, or having had bilateral ovariectomy), who did not use any hormone replacement therapy at the time of blood donation and who had no previous diagnosis of cancer (except nonmelanoma skin cancer). Case subjects were 677 women who developed breast cancer (614 invasive tumors, 63 carcinomas *in situ*) after their recruitment into the EPIC study and blood donation, and before the end of the study period, for each study center defined by the latest end-date of complete follow-up (overall, between June 1998 and December 2000). For each case subject, 2 control subjects were chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Matching characteristics were as follows: study center where the subjects were enrolled in the cohort, age at blood donation ($\pm$6 months), time of the day of blood collection and fasting status. A total of 1,309 controls were identified. Only cases and matched controls who had no missing values for hormone measurements were retained. A total of 613 cases and 1,139 matched controls were, therefore, included in the present study. A detailed description of numbers of breast-cancer cases and controls by study center is given elsewhere.[21]

Laboratory analyses

All assays were conducted on serum samples at the Hormone Laboratory at the International Agency for Research on Cancer in Lyon, France. E_2, E_1 and Δ_4 were measured by direct double-antibody radioimmunoassays from DSL (Diagnostic Systems Laboratories, Texas), while T and DHEAS were measured by direct radioimmunoassays from Immunotech (Marseille, France) and SHBG was measured by a direct ''sandwich'' immunoradiometric assay (Cis-Bio, Gif-sur-Yvette, France). Mean intrabatch and interbatch coefficients of variation were 5.8 and 13.1%, respectively, for E_2 (at a concentration of 250 pmol/l), 10.2 and 12.6% for E_1 (at 75 pmol/l), and 4.8 and 18.9% for Δ_4 (at 1.40 nmol/l), 10.8 and 15.3% T (at 1.40 nmol/l), 7.0 and 12.4%, respectively, for DHEAS (at 1.60 μmol/l) and 8.0 and 16.5% for SHBG (at 40 nmol/l). The different assays for hormone analyses were chosen on the basis of a comparative validation study that was published previously.[23] Serum concentrations of fT and fE_2—i.e., the fractions of hormones not linked to binding proteins in blood—were calculated from the absolute concentrations of the 2 steroids and SHBG using mass action equations, assuming a constant serum albumin concentration of 43 g/l.[24]

Statistical analyses

BMI was calculated as weight in kilograms divided by the square of height in meters, and categorized as $<$23.0, 23.0–25.0, 25.1–27.1, 27.2–30.2 and $>$30.2 kg/m^2, on the basis of the distribution of the control subjects. As well, waist and hip were categorized as $<$75.0, 75.1–81.0, 81.1–86.0, 86.1–93.0 and $>$93.0 cm, and $<$96.0, 96.1–101.0, 101.1–105.0, 105.1–111.0 and $>$111.0 cm, respectively. All hormone concentrations were log-transformed to normalize their distributions.

Statistical significance of case–control differences in anthropometric measures was evaluated by paired comparisons (*t*-tests) of case values versus the average values of the 2 matched controls, in each case–control set. For categorical variables, a chi-square test was used. Pearson correlation coefficients for relationships among hormones and anthropometric measures were calculated adjusting for age, case–control status and laboratory batch.

Odds ratios (ORs) for disease of different anthropometric measures were calculated by conditional logistic regression models, where BMI, waist, hip and WHR were examined as continuous variables and by quintiles. The quintiles were based on the distributions of controls of all EPIC centers combined. Likelihood ratio

TABLE I – BASELINE CHARACTERISTICS BY CASE/CONTROL STATUS

	Mean (5th percentile to 95th percentile)		p^1
	Cases (n = 613)	Controls (n = 1,139)	
BMI (kg/m^2)	27.2 (21.1–35.9)	26.9 (20.5–35.6)	0.17
HIP (cm)	105.1 (91.4–121.5)	104.2 (91.0–122.0)	0.06
WAIST (cm)	85.6 (69.0–105.0)	84.5 (69.0–105.0)	0.08
WHR	0.81 (0.71–0.92)	0.81 (0.71–0.91)	0.54
Age at menarche	13.2 (11.0–16.0)	13.3 (11.0–16.0)	0.04
Age at menopause	49.4 (41.0–55.0)	49.0 (40.0–55.0)	0.07
Number of full term pregnancies	2.4 (1.0–5.0)	2.6 (1.0–5.0)	0.09
Age at first full term pregnancy	26.1 (20.0–35.0)	25.7 (20.0–33.0)	0.08
Previous use of HRT	17.0%	18.3%	0.50
Previous use of OC	30.2%	34.4%	0.09
T (nmol/l)	1.28 (1.22–1.34)	1.16 (1.12–1.20)	0.0002
fT (pmol/l)	21.6 (20.5–22.9)	19.0 (18.3–19.8)	<0.0001
Δ_4 (nmol/l)	3.29 (3.14–3.45)	3.00 (2.90–3.10)	0.001
DHEAS (µmol/l)	2.16 (2.06–2.28)	1.94 (1.87–2.01)	0.0003
SHBG (nmol/l)	31.3 (29.8–32.9)	33.1 (32.0–34.3)	0.03
E_1 (pmol/l)	156.8 (151.5–162.3)	144.6 (141.0–148.4)	<0.0001
E_2 (pmol/l)	98.4 (95.0–102.0)	89.4 (87.1–91.8)	<0.0001
fE_2 (pmol/l)	2.61 (2.51–2.72)	2.34 (2.27–2.41)	<0.0001

[1]Paired t-test for case/control difference.

tests were used to assess linear trends in ORs with assigned quantitative scores 1–5 for the quintile categories. Confidence intervals (95%) were computed using the standard errors of the pertinent regression coefficients.

The effects of additional potential confounders (other than matching criteria, controlled for by design) were examined by including additional regression terms into the conditional logistic regression models. Potential confounders included age at menarche (missing, <12, 12, 13, 14, 15+ years), age at menopause (missing, ≤43, 44–47, 48–50, 51–52, 53–54, >54 years), number of full-term pregnancies (missing, nulliparous, 1, 2, 3, 4+), past use of exogenous hormones (oral contraceptives, hormone replacement therapy; as binary variables ''never/ever,'' missing), age at first full-term pregnancy (missing, nulliparous, ≤23, 24–25, 26–28, >28 years) and time since menopause (missing, 0–4, 5–14, 15+ years). Only 2 covariates that altered regression coefficients by at least 5%—number of full-term pregnancies, and age at first full-term pregnancy—were retained in the model as confounding variables.

To assess to which extent hormone concentrations might account for the association of BMI, waist, hip and WHR to breast cancer risk, ORs associated with anthropometric measures were calculated with and without adjustments for serum sex hormone concentrations as continuous variables. In addition, an analysis of variance adjusted for age and laboratory batch was used to estimate percentages of variance in hormone concentrations explained by BMI, waist and hip.

All statistical analyses were performed using the SAS statistical package, version 8.

Results

In this nested case–control study, average BMI, hip and waist circumferences were higher in breast cancer cases than in control subjects (27.2 *vs* 26. 9, p = 0.17, 105.1 *vs* 104.2, p = 0.06; and 85.6 *vs* 84.5, p = 0.08, respectively) (Table I), while there was no statistically significant difference between cases and controls for WHR. A total of 1,123 women were overweight (BMI >25) (421 cases and 702 controls), while a total of 373 women were obese (BMI >30) (128 cases and 245 controls). As reported previously,[21] breast cancer cases had higher serum levels of androgens and estrogens, and lower levels of SHBG, than the control subjects (all differences statistically significant) (Table I).

All 3 anthropometric indices of excess weight were highly correlated with each other after adjustment for age, case–control status and laboratory batch (Pearson's correlation coefficients ranging from r = 0.79–0.86, Table II). The WHR was also positively correlated to waist circumference (r = 0.72), less strongly with BMI (r = 0.42), and only very mildly with hip circumference (r = 0.14) (Table II). As for serum sex steroid concentrations, there were positive correlations between the 3 androgens (Pearson's correlations between r = 0.61 and 0.70), as well as between the 2 estrogens (E_1 and E_2; r = 0.55). Calculated values of fT and fE_2 were very highly correlated with the absolute concentrations of T and E_2 (r = 0.92 and r = 0.96, respectively), as previously reported.[24,25] SHBG was mildly negatively correlated to fT (r = −0.50) and fE_2 (r = −0.46). The androgens Δ_4 and T showed moderate positive correlations with serum estrogen concentrations (r = 0.42–0.55).

Anthropometric indices of adiposity (BMI, waist and hip circumferences, and WHR) showed mildly positive correlations with serum levels of E_1, E_2, fE_2 and fT (mostly between r = 0.10 and 0.34), and inverse correlations with SHBG (r = −0.41 to −0.30). By contrast, none of the anthropometric indices showed any significant correlation with serum concentrations of DHEAS and Δ_4, and only a very mild positive correlation could be observed between BMI, waist and hip, and concentrations of T (Pearson's r between 0.10 and 0.13). All correlations mentioned were virtually identical for breast cancer cases and control subjects separately (data not shown).

Mean serum levels of fE_2, E_2, E_1 and fT increased significantly with increasing quintile categories of BMI, waist and hip circumferences, whereas SHBG levels decreased (Table III). None of these trends were observed for categories of WHR (results not shown). Women in the highest BMI, hip and waist circumferences quintiles had on average 26% higher E_2, 51% higher fE_2, 22% higher E_1, 54% higher fT concentrations and 56% lower SHBG concentrations, compared to women in the lowest quintile. The percentage of variance explained in hormone concentrations by anthropometric indices of adiposity (expressed as continuous variable) varied from 2.9 to 11.0% for estrogens, from 0 to 6.5% for androgens and from 7.6 to 17.1% for SHBG.

Breast cancer risk was significantly related to BMI (OR = 1.22 [0.86–1.72], p_{trend} = 0.03, between top and bottom quintiles), waist circumference (OR = 1.58 [1.12–2.32], p_{trend} = 0.02) and hip circumference (1.40 [1.00–1.95], p_{trend} = 0.006) (Fig. 1), but was not significantly related to WHR (data not shown). These results are very similar to those seen previously in a full cohort analysis, among women who were not current HRT users.[6] On a continuous scale, a 5 kg/m^2 increase in BMI was associated with a 11% increase in breast cancer risk (Table IV),and similar increases

TABLE II – PEARSON CORRELATION COEFFICIENTS[1] [95% CI] AMONG SEX STEROIDS AND ANTHROPOMETRIC INDICES IN CASES AND CONTROLS

	fT	Δ_4	DHEAS	SHBG	E_1	E_2	fE_2	BMI	Waist	Hip	WHR
T	0.92	0.62	0.70	−0.08	0.55	0.49	0.46	0.10	0.13	0.13	0.06
	(0.91;0.92)	(0.59;0.65)	(0.68;0.72)	(−0.12; −0.03)	(0.52;0.58)	(0.46;0.53)	(0.43;0.50)	(0.05;0.15)	(0.08;0.17)	(0.08;0.17)	(0.01;0.10)
FT		0.58	0.67	−0.50	0.59	0.49	0.62	0.26	0.30	0.25	0.19
		(0.55;0.61)	(0.64;0.69)	(−0.54; −0.47)	(0.56;0.62)	(0.46;0.53)	(0.59;0.65)	(0.22;0.31)	(0.26;0.34)	(0.21;0.29)	(0.15;0.24)
Δ_4			0.61	−0.10	0.44	0.42	0.41	0.01	0.01	−0.03	0.05
			(0.58;0.64)	(−0.15; −0.05)	(0.40;0.48)	(0.38;0.46)	(0.37;0.44)	(−0.04;0.06)	(−0.04;0.05)	(−0.08;0.01)	(−0.00;0.09)
DHEAS				−0.14	0.52	0.52	0.51	0.03	0.05	0.03	0.04
				(−0.19; −0.09)	(0.49;0.56)	(0.48;0.55)	(0.47;0.54)	(−0.02;0.07)	(−0.00;0.09)	(−0.01;0.08)	(−0.01;0.08)
SHBG					−0.25	−0.15	−0.46	−0.38	−0.41	−0.30	−0.32
					(−0.29; −0.21)	(−0.19; −0.10)	(−0.50; −0.42)	(−0.42; −0.34)	(−0.45; −0.37)	(−0.34; −0.25)	(−0.36; −0.28)
E_1						0.55	0.57	0.27	0.26	0.22	0.16
						(0.52;0.58)	(0.53;0.60)	(0.23;0.31)	(0.21;0.30)	(0.18;0.27)	(0.12;0.21)
E_2							0.96	0.22	0.23	0.23	0.10
							(0.95;0.96)	(0.17;0.26)	(0.18;0.27)	(0.19;0.27)	(0.05;0.14)
FE_2								0.33	0.34	0.31	0.19
								(0.28;0.37)	(0.30;0.38)	(0.27;0.35)	(0.15;0.24)
BMI									0.86	0.85	0.42
									(0.85;0.87)	(0.84;0.86)	(0.38;0.46)
Waist										0.79	0.72
										(0.77;0.81)	(0.69;0.74)
Hip											0.14
											(0.09;0.19)

[1]Adjusted for age, case–control status and laboratory batch.

in risk (12 and 14%, respectively) were observed for a 10 cm increase in waist circumference, or hip circumference. For none of these relationships was there any clear heterogeneity between EPIC study centers or countries.

As reported in detail in a separate publication,[21] breast cancer risk showed approximate 2-fold relative risks between the highest and lowest quintile levels of serum fE_2 and fT, and slightly weaker associations also with serum levels of E_2, E_1, T, DHEAS and Δ_4. Adjustments for serum androgen levels (T, Δ_4, DHEAS) as continuous variables did not substantially alter the associations of breast cancer risk with any of the anthropometric indices of adiposity (Table IV). An adjustment for fT, however, did substantially reduce relative risks, and this was also the case for adjustments for E_1, E_2, fE_2 and SHBG. The ORs of breast cancer decreased most strongly after adjustment for all estrogens from 1.22 [0.86–1.72] to 0.92 [0.64–1.36] for highest *vs.* lowest quintiles of BMI, and from 1.58 [1.12–2.32] to 1.26 [0.86–1.84], and from 1.40 [1.00–1.95] to 1.14 [0.80–1.62], respectively, between the extreme quintiles of waist and hip circumferences (Fig. 1). Similar reductions were also seen for the estimated relationships of breast cancer risk with the anthropometric indices expressed as continuous variables (Table IV).

Discussion

We have analyzed the interrelationships between selected anthropometric measures, endogenous sex hormone levels, and postmenopausal breast cancer risk, in a large prospective study (nested case–control design). The results of this analysis suggest that the increase in breast cancer risk with increasing adiposity can at least in part be attributed to increases in endogenous sex hormone concentrations, especially E_1, E_2, fE_2 and to a lesser extent fT, but not to increases in total serum androgen concentrations (DHEAS, Δ_4, T).

In agreement with the full cohort analyses in EPIC published recently,[6] as well as with other previous studies,[2,3] this nested case–control study in postmenopausal women showed direct associations of breast cancer risk with BMI, and waist and hip circumferences. Consistent with the results from previous studies, including the large pooled reanalysis of 8 other prospective cohort studies on BMI, endogenous sex hormones and breast cancer risk,[3] we observed an ~11% increase in beast cancer risk for a 5 kg/m^2 increase in BMI. Similar increases in risk were observed for a 10 cm increase in either waist or hip circumference.

Quintiles of BMI showed a nonlinear relationship with breast cancer risk, with the highest relative risk in the fourth quintile level (a BMI of up about 27.2–30.2 kg/m^2), and a somewhat lesser increase in risk for BMI levels exceeding 30 kg/m^2. Women in the highest quintile of BMI had a risk of breast cancer that was 40% lower than women in the fourth quintile. A similar nonlinear relationship was also observed in 2 different pooled analyses of prospective studies.[2,3] It has been previously suggested that the moderate decline in the relative risk for the highest BMI category could be the remnant of a protective effect of severe weight excess (BMI >30 kg/m^2) during premenopausal years.[3]

Waist and hip circumferences were more strongly related to breast cancer risk than BMI. This could be due to the fact that BMI does not allow for variations in the lean-to-fat mass proportion in the body, while especially waist circumference correlates with the amount of abdominal (visceral and subcutaneous) fat[26–28]—the adipose tissue component most strongly associated with insulin resistance, hyperinsulinemia and lower SHBG. On the other hand, in the current study, as in some,[29,30] but not all,[31] previous prospective studies, no association with breast cancer risk was found for WHR, an index of the relative accumulation of abdominal compared to gluteal fat. In our study, waist and hip circumferences were both highly related to risk, and also highly correlated to each other, so that calculating the ratio between the 2 measures could have attenuated the associations with cancer risk.

TABLE III – GEOMETRIC MEANS (95% CI) OF SEX HORMONES BY ANTHROPOMETRIC MEASURES IN 1,139 HEALTHY POSTMENOPAUSAL WOMEN, ADJUSTED FOR AGE AND LABORATORY BATCH

Anthropometry	*N*	T (nmol/l)	fT (pmol/l)	Δ_4 (nmol/l)	DHEAS (μmol/l)	E_2 (pmol/l)	fE_2 (pmol/l)	E_1 (pmol/l)	SHBG (nmol/l)
BMI									
<23.0	227	1.10 (1.02–1.18)	15.1 (13.9–16.4)	2.87 (2.65–3.09)	1.88 (1.73–2.05)	81.8 (77.7–86.1)	1.94 (1.83–2.05)	131.2 (125.0–137.6)	44.5 (41.5–47.7)
23.0–25.0	230	1.13 (1.06–1.22)	17.4 (16.0–18.8)	2.92 (2.70–3.14)	1.96 (1.80–2.13)	82.1 (78.0–86.4)	2.06 (1.95–2.18)	134.4 (128.2–140.9)	38.3 (35.7–41.0)
25.1–27.1	226	1.14 (1.06–1.22)	18.5 (17.0–20.1)	3.06 (2.83–3.30)	1.88 (1.73–2.05)	87.3 (82.9–92.0)	2.27 (2.14–2.40)	142.1 (135.4–149.0)	34.5 (32.2–37.0)
27.2–30.2	228	1.17 (1.09–1.26)	20.6 (19.0–22.4)	3.17 (2.93–3.42)	2.02 (1.85–2.19)	92.8 (88.1–97.7)	2.51 (2.38–2.66)	148.3 (141.4–155.5)	29.6 (27.6–31.7)
>30.2	228	1.23 (1.14–1.32)	23.8 (21.9–25.9)	3.07 (2.84–3.32)	1.93 (1.77–2.11)	105.2 (99.8–110.9)	3.01 (2.84–3.20)	165.1 (157.3–173.5)	24.3 (22.6–26.1)
p for trend		0.04	<0.0001	0.08	0.57	<0.0001	<0.0001	<0.0001	<0.0001
% variance explained by BMI in the model		0.4	5.4	0.4	0.2	5.1	11.0	4.5	12.7
Waist									
<75.0	221	1.12 (1.04–1.21)	15.1 (13.9–16.5)	3.02 (2.79–3.26)	1.93 (1.77–2.10)	83.1 (78.8–87.5)	1.95 (1.84–2.06)	133.2 (126.8–139.8)	47.4 (44.2–50.8)
75.1–81.0	218	1.07 (1.00–1.16)	16.6 (15.2–18.0)	2.81 (2.59–3.04)	1.78 (1.63–1.94)	81.3 (77.1–85.7)	2.05 (1.93–2.17)	134.0 (127.5–140.8)	37.9 (35.3–40.6)
81.1–86.0	219	1.14 (1.06–1.23)	18.0 (16.5–19.6)	3.02 (2.79–3.26)	1.89 (1.73–2.06)	85.5 (81.1–90.2)	2.19 (2.07–2.32)	137.8 (131.3–144.7)	36.2 (33.8–38.8)
86.1–93.0	244	1.19 (1.11–1.28)	20.9 (19.4–22.7)	3.26 (3.02–3.51)	2.10 (1.94–2.28)	93.7 (89.1–98.5)	2.55 (2.42–2.70)	151.6 (144.7–158.8)	29.1 (27.2–31.1)
>93.0	237	1.23 (1.14–1.32)	24.3 (22.4–26.4)	2.95 (2.74–3.19)	1.97 (1.81–2.15)	103.9 (98.7–109.4)	2.98 (2.82–3.16)	161.8 (154.2–169.8)	23.4 (21.8–25.0)
p for trend		0.02	<0.0001	0.41	0.14	<0.0001	<0.0001	<0.0001	<0.0001
% variance explained by waist in the model		0.7	6.5	0.7	0.7	4.9	11.0	4.0	17.1
Hip									
<96.0	200	1.09 (1.01–1.18)	15.9 (14.5–17.4)	2.99 (2.75–3.25)	1.96 (1.79–2.15)	82.3 (77.9–87.0)	1.99 (1.87–2.12)	133.6 (126.8–140.7)	40.4 (37.4–43.7)
96.1–101.0	243	1.11 (1.04–1.19)	17.0 (15.7–18.4)	2.96 (2.75–3.19)	1.85 (1.70–2.00)	83.7 (79.6–88.0)	2.10 (1.98–2.22)	137.6 (131.3–144.2)	38.2 (35.6–40.9)
101.1–105.0	210	1.23 (1.14–1.32)	19.2 (17.7–21.0)	3.00 (2.77–3.25)	2.02 (1.85–2.21)	88.7 (84.0–93.6)	2.27 (2.14–2.41)	140.0 (133.2–147.2)	36.2 (33.6–39.0)
105.1–111.0	235	1.13 (1.05–1.21)	19.6 (18.1–21.3)	3.05 (2.83–3.29)	1.94 (1.78–2.10)	89.6 (85.1–94.3)	2.43 (2.29–2.57)	145.6 (138.9–152.7)	30.3 (28.2–32.5)
>111.0	251	1.21 (1.13–1.29)	22.5 (20.8–24.4)	3.05 (2.83–3.28)	1.93 (1.77–2.09)	102.4 (97.4–107.7)	2.87 (2.71–3.03)	160.4 (153.1–168.1)	26.2 (24.5–28.1)
p for trend		0.08	<0.0001	0.56	0.88	<0.0001	<0.0001	<0.0001	<0.0001
% variance explained by hip in the model		0.7	3.4	0.0	0.2	3.7	7.4	2.9	7.6

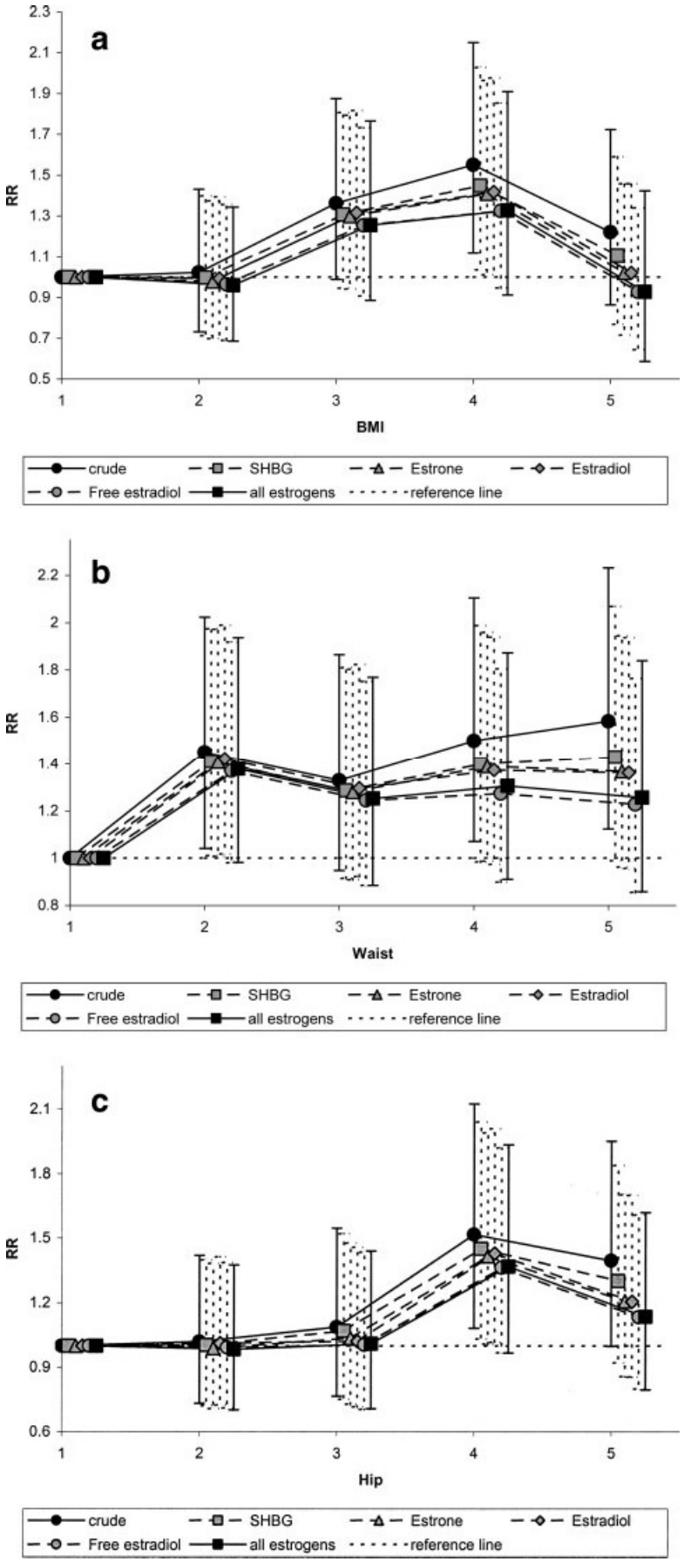

Figure 1 – Relative risk of breast cancer by quintiles of BMI, waist and hip in cancer cases and their matched controls (adjusted for number of full-term pregnancies and age at full-term pregnancy), with further adjustment for sex-steroids. (*a*) BMI adjusted for estrogens and SHBG; (*b*) Waist adjusted for estrogens and SHBG; (*c*) Hip adjusted for estrogens and SHBG.

TABLE IV – RELATIVE RISKS OF BREAST CANCER ASSOCIATED WITH ANTHROPOMETRIC MEASUREMENTS, WITH FURTHER ADJUSTMENT FOR STEROID LEVELS

	RR (95% CI) per 5 kg/m^2 increase in BMI	RR (95% CI) per 10 cm increase in waist	RR (95% CI) per 10 cm increase in hip
Adjusted model[1]	**1.11 (0.99–1.25)**	**1.12 (1.02–1.24)**	**1.14 (1.02–1.27)**
Further adjusted for[1]**:**			
Testosterone	1.10 (0.98–1.24)	1.12 (1.01–1.23)	1.13 (1.01–1.27)
Free testosterone	1.05 (0.94–1.19)	1.07 (0.97–1.19)	1.10 (0.98–1.23)
Androstenedione	1.12 (1.00–1.26)	1.13 (1.02–1.25)	1.16 (1.03–1.29)
DHEAS	1.11 (0.99–1.25)	1.12 (1.02–1.24)	1.15 (1.03–1.28)
All androgens	**1.07 (0.94–1.22)**	**1.09 (0.98–1.22)**	**1.12 (0.99–1.26)**
SHBG	1.07 (0.94–1.21)	1.08 (0.97–1.21)	1.11 (0.99–1.24)
Estrone	1.04 (0.92–1.17)	1.07 (0.96–1.18)	1.08 (0.97–1.21)
Estradiol	1.03 (0.91–1.16)	1.06 (0.96–1.17)	1.07 (0.96–1.20)
Free estradiol	0.99 (0.87–1.12)	1.02 (0.92–1.14)	1.05 (0.93–1.18)
All estrogens	**0.99 (0.87–1.13)**	**1.03 (0.92–1.15)**	**1.05 (0.93–1.18)**

[1]Adjusted for number of full-term pregnancies and age at first full-term pregnancy.

The anthropometric indices of adiposity showed direct associations with serum levels of estrogens, fE_2 and fT, and an inverse association with SHBG, both among breast cancer cases or control subjects. The increase in levels of E_1, E_2 and fE_2 with increasing adiposity can be explained by the fact that, in postmenopausal women, estrogens are mainly produced in the adipose tissue by aromatization of androgens.[14] Increasing adiposity was also associated with an increase in serum fT concentrations (but not with total T concentrations); this association might be explained by the relatively high positive correlation of fT with fE_2, and by the inverse correlation of fT with SHBG. For each of these hormonal variables, adjustments led to a reduction in relative risk estimates of breast cancer with respect to adiposity indices, even though this reduction was less strong for hip circumference.

As in the pooled reanalysis published by the Endogenous Hormones and Breast Cancer Collaborative Group,[3] the strongest reduction in relative risk estimates was obtained when an adjustment was made for fE_2 concentrations. Interestingly, however, none of the adiposity indices showed any association with total serum concentrations of DHEAS, Δ_4 or T, and thus there was no evidence that any of these 3 androgens could potentially mediate effects of adiposity on breast cancer risk.

Our data showed associations of BMI with estrone, total and free estradiol, and free testosterone, but no clear relationship with DHEAS, androstenedione or total testosterone. In the nested case–control study,[21] elevated serum levels of all steroids (androgens and estrogens) were associated with an increase in risk of breast cancer (an approximate 2-fold increase), while serum concentrations of SHBG were associated with a decrease in risk. Adjusting for BMI (as an index for adiposity) had little effect on relative risk estimates of breast cancer for different levels of androgens, estrogens or SHBG, suggesting a possible causal role of sex steroids in breast cancer independently of obesity. Conversely, adjusting for total serum androgens did not alter relative risks for BMI much, whereas adjustments for total or free estrogens did substantially reduce the BMI-risk relationship.

One major strength of this study is its prospective design, which eliminates the possibility that serum hormone concentrations were altered by the presence of a large tumor, or due to the diagnosis and/or treatment of cancer. In addition, the large number of cases and controls for which hormone measurements and anthropometric indexes were available (613 cases and 1,139 controls) makes it the largest prospective study ever published on breast cancer risk, hormones and anthropometry. All hormones were measured in the same laboratory, using the same, well-validated assays for all study subjects.

The percentage of variance in hormone levels explained by adiposity indices was estimated to be between 3 and 5% for E_1 and E_2, up to 17% for SHBG and about 4–11% for fT or fE_2. These percentages are relatively modest, indicating that the serum levels of these hormones are also influenced by other factors than adiposity. It is likely, however, that the true percent of variance in hormone levels due to varying degrees of adiposity is, in reality, substantially higher than our estimates might suggest. First, BMI is considered a surrogate measure of body fat. Studies relating BMI to body fat percent measured by under-water weighing have shown a correlation of only about 0.5–0.6.[1] Second, our study was limited by the fact that we had hormone measurements for only a single serum sample per study participant, which may not perfectly reflect average serum hormone concentrations over the somewhat longer term (although several studies have shown fairly high correlations [>0.80] between hormone measurements in serum samples taken from postmenopausal women, over intervals of 1 year or longer[3,32,33]).

Taken together, our data suggest that the relationship of adiposity with increased breast cancer risk in postmenopausal women could in part be explained by increases in serum levels of endogenous sex steroids, notably E_1, E_2, fE_2 and fT. These hormonal factors may well be modifiable by weight control, and possibly also by diet[34] and physical exercise,[35] as reported previously by other research groups; therefore, they are very interesting for prevention of breast cancer. However, adiposity indices showed no clear relationships with serum total androgen concentrations.

Further studies will be needed to understand which factors, related to lifestyle, could be determinants of the increased serum androgen levels observed in women who subsequently develop breast cancer, and to examine the possible role of other metabolic and/or hormonal factors that may link excess weight and adiposity to breast tumor development.

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