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# C-peptide, IGF-I, sex-steroid hormones and adiposity: a cross-sectional study in healthy women within the European Prospective Investigation into Cancer and Nutrition (EPIC)

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**Abstract**

*Objectives*: The risk of some cancers is positively associated with body weight, which may influence circulating levels of sex-steroid hormones, insulin and IGF-I. Interrelationships between these hormones and the associations with adiposity were evaluated in healthy women participating in the European Prospective Investigation into Cancer and Nutrition (EPIC).

*Methods*: A cross-sectional analysis was performed on anthropometric and hormonal data from 743 pre- and 1217 postmenopausal women. Body mass index (BMI) and waist circumference were used as indicators of adiposity. C-peptide, Insulin Growth Factor (IGF)-I, Insulin Growth Factor binding protein (IGFBP)-3, androgens, estrogens and sex hormone binding globulin (SHBG) were measured by immunoassays; free sex steroid concentrations were calculated.

*Results*: BMI and waist circumference were positively correlated with estrogens in postmenopausal women and with C-peptide, free testosterone and inversely with SHBG in all women. C-peptide and IGF-I were inversely

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correlated with SHBG, and positively with free sex steroids in postmenopausal women. IGF-I was positively associated with postmenopausal estrogens and androgen concentrations in all women.
*Conclusions*: Sex-steroid concentrations appear to be regulated along several axes. Adiposity correlated directly with estrogens in postmenopausal women and with insulin, resulting in lower SHBG and increased levels of free sex steroids. Independent of adiposity and insulin, IGF-I was associated with decreased SHBG levels, and increased concentrations of androgens and postmenopausal estrogens.

## Introduction

Excess body weight has proved to be an important risk factor for cancer, including postmenopausal breast cancer and cancers of the kidney, pancreas, colon and endometrium [1–4]. Obesity-related alterations in the production and metabolism of hormones like insulin, insulin-like growth factor (IGF)-I and sex-steroid hormones have been proposed to explain the link between adiposity and cancer risk [5–8].

*In vitro* studies have shown that insulin, IGF-I and sex-steroid hormones all promote cell growth and proliferation, and serum levels of these hormones have been associated with cancer risk [5, 9–11]. Evaluation of interrelationships between these hormones and their associations with adiposity may therefore provide information about the role of hormones in the association between excess weight and cancer risk. Fasting serum insulin concentrations are continuously and linearly related to obesity [7] and in postmenopausal women, increased estrogen levels were observed in heavier women [5]. Both insulin and IGF-I have been shown to stimulate sex-steroid synthesis and inhibit sex hormone binding globulin (SHBG) production [6]. However, the cross-sectional associations between obesity, IGF-I and sex-steroid hormones in healthy women remain unclear, and insulin was not included in most studies [12–15].

The present cross-sectional study aims to explore the interrelationships between insulin, IGF-I and sex-steroid hormones, and the associations with adiposity, in a large population sample. Anthropometric and hormonal data were obtained from 1960 (743 pre- and 1217 postmenopausal) women participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) [16]. BMI and waist circumference were used as anthropometric indices of adiposity, and the hormonal data included C-peptide (a marker for insulin production), IGF-I, IGF binding protein (IGFBP-3), androgens (free and total testosterone, androstenedione, dehydroepiandrosterone (DHEAS)), estrogens (estrone, free and total estradiol) and SHBG.

## Materials and methods

### *Study population*

This cross-sectional study included pre- and postmenopausal women participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). The EPIC study is a multi-centre prospective cohort study including over 500,000 participants, men and women, recruited in 23 centres located in ten Western European countries between 1992 and 2001 [16]. At baseline, blood samples and information about lifestyle and dietary variables were collected, and anthropometric measurements were performed. All participants signed an informed consent agreement to participate in the study and approval was obtained from the ethical review boards of the International Agency for Research on Cancer (IARC, Lyon, France) and all local institutions participating in EPIC.

The women included in this study were selected as controls in a breast cancer case-control study, nested within EPIC, and were from eight of the ten participating countries: Greece (n = 66), Spain (n = 356), Italy (n = 504), France (n = 117), Germany (n = 108), the Netherlands (n = 466), the United Kingdom (n = 284) and Denmark (n = 59). Because the breast cancer study investigated the role of endogenous hormone levels on subsequent risk of developing breast cancer, women who were using exogenous hormones (oral contraceptives or hormone replacement therapy) at the date of blood collection were excluded. The control subjects were matched with breast cancer cases on study center, age, follow-up time, time of the day at blood collection, fasting status and, for premenopausal women, menstrual cycle phase.

Women were considered postmenopausal when they reported the absence of any menses over the previous 12 months or a bilateral ovariectomy. When questionnaire data were missing or incomplete, or when hysterectomy without ovariectomy was reported, women were considered premenopausal when they were younger than 42 years, and postmenopausal

when they were older than 55 years at the date of blood collection. Estrogen levels in premenopausal women are highly variable, depending on the phase of the menstrual cycle in which the blood sample was taken. To facilitate controlling for this variation, only women of whom menstrual cycle information was available (n = 568) were included in the analyses of correlations between estrogens and other variables. Phase of menstrual cycle was determined using either 'forward' dating, counting from the women's reported dates of the start of their last menses, or by 'backward' dating, counting backward from the date of the start of their next menses, reported on a prestamped postcard that women sent back after their visit to the recruitment centre for blood donation. Preference was given to the backward dating because the length of the second (luteal) half of the cycle is generally more constant between women than the first half (follicular phase). When the date of blood donation differed more than 40 days from a woman's nearest reported date of menstrual periods (either last or next), menstrual cycle phase was left undetermined. To combine women whose dates of menstrual cycle were based on either forward or backward dating, a standard length of cycle of 29 days was assumed to apply to all women. This length corresponded to the mean cycle length in EPIC women who provided dates of both last and next menses.

Height, weight and waist circumference were measured in most study subjects and women having only self-reported anthropometric information (UK cohorts) were excluded from the analysis of anthropometry and hormone levels. Waist circumference is an indicator of fat distribution, and body mass index (BMI), calculated from height and weight measurements, was used as an indicator of overall adiposity. C-peptide was included as a marker of insulin production. Because insulin production is highly influenced by food intake, only samples obtained after an overnight fast (n = 757) were included in the analyses of correlations between C-peptide and other variables.

From the original control group (n = 2278), 15 women with a diagnosis of cancer (except non-melanoma skin cancer) and 14 other women taking insulin as a medication for diabetic conditions were excluded from the present analysis. Also excluded were 192 women whose menopausal status was equivocal, 93 women (all recruited in Oxford, UK) who had only self-reported or missing values for anthropometric measurements, and four women who had missing values for anthropometric data. The present cross-sectional analysis includes 1960 women, of whom 743 were premenopausal and 1217 were postmenopausal.

### *Laboratory analyses*

Blood samples were collected according to a standardized protocol. In all countries except Denmark, blood samples were fractionated and aliquoted in plastic straws containing 0.5 ml of plasma, serum, erythrocytes or buffy coat. Samples were stored locally and centrally at IARC in liquid nitrogen (−196 °C). In Denmark, blood fractions were stored in 1 ml tubes and stored only locally, in nitrogen vapour (−150 °C). All laboratory measurements were performed at the Hormone Laboratory at IARC.

C-peptide, androstenedione, estrone and estradiol in post-menopausal women were measured by radioimmunoassay from Diagnostic Systems Laboratories (DSL, Webster, TX, USA). Testosterone, DHEAS and progesterone were measured by radioimmunoassay with reagents from Immunotech (Marseille, France). Estradiol in pre-menopausal women was measured by radioimmunoassay from DiaSorin (Saluggia, Italy). SHBG was measured by immunoradiometric assays with reagents from Cis-bio (Gif-sur-Yvette, France). IGF-I and IGFBP-3 were measured by enzyme-linked immunosorbent assays (ELISA, DSL). The protocol for IGF-I included an acid–ethanol precipitation step to remove IGF-binding proteins. The overall mean intra- and inter- batch coefficients of variation were 9.9% and 15.3%, respectively, for testosterone, 6.2% and 12.4% for DHEAS, 5.3% and 18.9% for androstenedione, 6.4% and 12.6% for estrone, 3.2% and 7.2% for estradiol in premenopausal women, 5.8% and 13.1% for estradiol in postmenopausal women, and 7.5% and 16.5% for SHBG, 6.2% and 16.2%, respectively, for IGF-I and 7.2% and 9.7% for IGFBP-3.

Free testosterone and estradiol were calculated on the basis of total testosterone, total estradiol and SHBG concentrations, as described previously [17].

### *Statistical analysis*

All hormone data were log-transformed to approach a normal distribution. Univariate regression analyses were performed to compare geometric mean levels of age, anthropometry and hormone levels between pre- and postmenopausal women.

Pearson correlations adjusted for age, laboratory batch and BMI in quintiles were calculated for pre- and postmenopausal women separately to assess relationships of age and anthropometry with hormone levels, and between hormone levels. Furthermore, multivariate regression analyses were performed to compare geometric means of hormone levels by quintiles of IGF-I and tests for linear trend were performed by assigning equally spaced quantitative scores to the successive

IGF-I categories and treating the variables as continuous in the regression model. Age, laboratory batch and BMI (in quintiles) were included as covariates in the model.

The laboratory assays were performed in separate batches, according to menopausal status and EPIC recruitment centre. Therefore, adjustments for study centre were redundant after adjustment for analytical batch. All the analyses on estrogen levels were also adjusted for phase of the menstrual cycle in five categories: 'early follicular' (days 0–7 of the cycle), 'late follicular' (days 8–11), 'peri-ovulatory' (days 11–16), 'midluteal' (days 20–24) and 'other luteal' (days 17–19 or 25–40).

All tests of significance were two-sided and $p$-values of $< 0.05$ were considered statistically significant. All analyses were performed using the Statistical Analysis System software program (SAS institute, Cary, NC, USA).

## Results

Mean age was 45.7 in premenopausal and 60.2 in postmenopausal women (mean time since menopause: 9.3 years) (Table 1). Postmenopausal women had significantly higher BMI and waist circumference than premenopausal women. C-peptide levels were also higher in postmenopausal women while IGF-I, sex steroid and SHBG levels were all lower than in premenopausal women. Pre- and postmenopausal women had similar levels of IGFBP-3. After adjustment for age, IGF-I levels were comparable between the two groups, but the other hormone concentrations remained significantly different between pre- and postmenopausal women (data not shown).

Age correlated significantly inverse with IGF-I ($r = -0.27$ for premenopausal women and $r = -0.14$ for postmenopausal women), DHEAS ($r = -0.09$ and $r = -0.13$), with IGFBP-3 in premenopausal women and with estradiol in postmenopausal women (Table 2).

BMI and waist circumference correlated positively with fasting C-peptide levels (correlations with BMI and waist circumference in pre- and postmenopausal women ranged from 0.50 to 0.57) and free testosterone (range from 0.24 to 0.29) and inversely with SHBG (range from −0.37 to −0.43). Positive correlations between calculated indices of free estradiol and adiposity were only observed in the postmenopausal group ($r = 0.32$ for BMI and $r = 0.33$ for waist). IGF-I did not show any linear correlation with BMI or waist circumference in either group, although a nonlinear pattern was observed with peak levels in the third quintile of BMI (data not shown), as reported previously [12, 18, 19]. IGFBP-3 correlated weakly positively with BMI ($r = 0.09$) and waist circumference ($r = 0.11$). Correlations with hormone levels were systematically slightly stronger for waist circumference than for BMI, but not statistically significant.

For total concentrations of sex-steroid hormones (either bound or unbound to SHBG), BMI and waist circumference correlated weakly with testosterone (range from 0.06 to 0.09) and with DHEAS only in premenopausal women ($r = 0.11$ and $r = 0.15$, for BMI and waist circumference, respectively), and with estrone ($r = 0.23$ and $r = 0.21$) and estradiol ($r = 0.21$ and $r = 0.22$) in postmenopausal women.

Among the three androgens (testosterone, androstenedione and DHEAS), Pearson correlations were strong in both pre- and postmenopausal women (range from 0.54 to 0.71), as well as between the two estrogens

*Table 1.* Geometric means (95% CI) of age, anthropometry and hormone levels by menopausal status

| | Premenopausal women | | | Postmenopausal women | | | $p$ |
|---|---|---|---|---|---|---|---|
| | N | Geometric mean | (95% CI) | N | Geometric mean | (95% CI) | |
| Age (yr) | 743 | 45.7 | (45.4–46.0) | 1217 | 60.2 | (59.9–60.6) | < 0.001 |
| Time since menopause (yr) | – | – | – | 1153 | 9.3 | (8.9–9.7) | |
| BMI (kg/m$^2$) | 743 | 25.1 | (24.8–25.4) | 1217 | 26.5 | (26.3–26.8) | < 0.001 |
| Waist (cm) | 743 | 79.1 | (78.4–79.8) | 1215 | 84.0 | (83.4–84.6) | < 0.001 |
| C-peptide (nmol/l) | 350 | 0.82 | (0.79–0.85) | 407 | 0.91 | (0.88–0.94) | < 0.001 |
| IGF-1 (nmol/l) | 740 | 33.5 | (32.7–34.3) | 1215 | 27.3 | (26.8–27.8) | < 0.001 |
| IGFBP-3 (nmol/l) | 709 | 118 | (115–122) | 1157 | 118 | (115–120) | 0.675 |
| Testosterone (nmol/l) | 733 | 1.5 | (1.4–1.5) | 1196 | 1.1 | (1.1–1.2) | < 0.001 |
| Free testosterone (pmol/l) | 732 | 20.5 | (19.5–21.6) | 1196 | 18.6 | (17.9–19.4) | 0.003 |
| Androstenedione (nmol/l) | 740 | 4.6 | (4.4–4.8) | 1195 | 3.0 | (2.9–3.1) | < 0.001 |
| DHEAS ($\mu$mol/l) | 742 | 3.2 | (3.0–3.3) | 1200 | 1.9 | (1.9–2.0) | < 0.001 |
| SHBG (nmol/l) | 741 | 45.1 | (43.2–47.0) | 1217 | 33.5 | (32.4–34.6) | < 0.001 |
| Estrone (pmol/l) | 568 | 352 | (337–368) | 1173 | 145 | (141–150) | < 0.001 |
| Estradiol (pmol/l) | 568 | 289 | (275–303) | 1214 | 89.3 | (86.3–92.4) | < 0.001 |
| Free estradiol (pmol/l) | 569 | 6.9 | (6.6–7.3) | 1214 | 2.3 | (2.3–2.4) | < 0.001 |

*Table 2.* Pearson correlations (95% CI) of age with hormone and SHBG levels

| | Premenopausal women | | | Postmenopausal women | | |
|---|---|---|---|---|---|---|
| | Age[a] | BMI[b] | Waist[b] | Age[a] | BMI[b] | Waist[b] |
| C-peptide | −0.03 (−0.14;0.07) | 0.50 (0.42;0.57) | 0.57 (0.50;0.64) | −0.01 (−0.11;0.08) | 0.50 (0.43;0.57) | 0.54 (0.47;0.61) |
| IGF-1 | −0.27 (−0.33;−0.20) | −0.01 (−0.08;0.06) | 0.01 (−0.06;0.08) | −0.14 (−0.20;−0.09) | −0.03 (−0.08;0.03) | −0.04 (−0.10;0.01) |
| IGFBP-3 | −0.10 (−0.17;−0.02) | 0.09 (0.02;0.17) | 0.11 (0.04;0.18) | −0.04 (−0.10;0.02) | 0.09 (0.03;0.14) | 0.11 (0.05;0.16) |
| Testosterone | −0.05 (−0.12;0.03) | 0.08 (0.01;0.15) | 0.09 (0.02;0.17) | −0.05 (−0.10;0.01) | 0.07 (0.01;0.13) | 0.06 (0.00;0.12) |
| Free testosterone | −0.06 (−0.13;0.02) | 0.26 (0.19;0.32) | 0.29 (0.22;0.35) | −0.06 (−0.12;−0.01) | 0.24 (0.19;0.30) | 0.25 (0.20;0.30) |
| Androstenedione | −0.07 (−0.14;0.01) | 0.01 (−0.06;0.08) | 0.03 (−0.04;0.10) | −0.05 (−0.10;0.01) | 0.03 (−0.03;0.09) | 0.01 (−0.05; 0.07) |
| DHEAS | −0.09 (−0.16;−0.02) | 0.11 (0.04;0.18) | 0.15 (0.08;0.22) | −0.13 (−0.19;−0.08) | 0.04 (−0.01;0.10) | 0.04 (−0.02;0.10) |
| SHBG | 0.04 (−0.03;0.11) | −0.37 (−0.44;−0.31) | −0.40 (−0.46;−0.34) | 0.04 (−0.02;0.09) | −0.39 (−0.44;−0.35) | −0.43 (−0.48;−0.39) |
| Estrone[c] | 0.05 (−0.03;0.13) | 0.07 (−0.02;0.15) | 0.05 (−0.03;0.14) | 0.00 (−0.06;0.06) | 0.23 (0.17;0.28) | 0.21 (0.16;0.27) |
| Estradiol[c] | 0.07 (−0.02;0.14) | −0.04 (−0.12;0.04) | −0.04 (−0.13;0.04) | −0.11 (−0.16;−0.05) | 0.21 (0.16;0.27) | 0.22 (0.17;0.27) |
| Free estradiol[c] | 0.06 (−0.02;0.14) | 0.04 (−0.04;0.13) | 0.05 (−0.04;0.13) | −0.09 (−0.15;−0.04) | 0.32 (0.27;0.37) | 0.33 (0.28;0.38) |

Adjusted for: [a] batch and BMI.
[b] age and batch.
[c] phase of menstrual cycle (premenopausal women).

($r = 0.74$ and $r = 0.65$, in pre- and postmenopausal women, respectively) (Table 3). Estradiol correlated positively with all androgens in postmenopausal women (range from 0.44 to 0.47), whereas in premenopausal women estradiol correlated with testosterone ($r = 0.20$) and androstenedione ($r = 0.30$) but not with DHEAS. Estrone, on the other hand, correlated positively with all androgens (range from 0.23 to 0.49) in both pre- and postmenopausal women.

SHBG was inversely correlated with the calculated indices of free testosterone ($r = -0.59$ in pre- and $r = -0.45$ in postmenopausal women) and DHEAS ($r = -0.24$ and $r = -0.16$), and weakly with total testosterone and androstenedione. Only in postmenopausal women was SHBG inversely correlated to estrone ($r = -0.16$), estradiol ($r = -0.12$) and free estradiol ($r = -0.38$). In premenopausal women, SHBG and estradiol were positively correlated ($r = 0.15$).

C-peptide and IGF-I correlated similarly, but independently, with most sex steroids and SHBG; the correlation between the two peptides was low ($r = 0.17$ in pre- and $r = 0.26$ in postmenopausal women, data not shown) and the associations of C-peptide and IGF-I with sex steroids did not change by adjustment for IGF-I or C-peptide, respectively (data not shown). Pearson correlations of C-peptide (Table 3) and IGF-I (Figures 1 and 2) with sex steroids were calculated and in addition, sex steroid concentrations were presented across quintiles of IGF-I, for which more data were available than for C-peptide, and corresponding trends were observed. Inverse correlations were observed of SHBG with C-peptide ($r = -0.29$ and $r = -0.42$) and IGF-I ($r = -0.28$ and $r = -0.23$), and consistent positive correlations were observed between peptide hormones and free sex steroids (range from 0.16 to 0.20) in postmenopausal women. In all women, IGF-I was positively correlated to both total (range from 0.10 to 0.18) and free androgens ($r = 0.23$ in premenopausal women and $r = 0.20$ in postmenopausal women). For C-peptide, a positive association was only observed with androstenedione in postmenopausal women ($r = 0.12$). Total estrogens were weakly correlated with IGF-I in postmenopausal women. An initially positive association between C-peptide and total estrogens in postmenopausal women ($r = 0.17$ for estrone and $r = 0.19$ for estradiol) diminished after adjustment for BMI.

IGFBP-3 was positively correlated with IGF-I ($r = 0.48$ in premenopausal and $r = 0.45$ in postmenopausal

*Table 3.* Pearson correlations[a] (95% CI) between C-peptide, sex-steroid hormones and SHBG

| | Testosterone | Free testosterone | Androstene-dione | DHEAS | SHBG | Estrone[b] | Estradiol[b] | Free estradiol[b] |
|---|---|---|---|---|---|---|---|---|
| *Premenopausal women* | | | | | | | | |
| C-peptide | −0.07 (−0.18;0.03) | 0.09 (−0.01;0.20) | −0.02 (−0.12;0.09) | 0.04 (−0.06;0.15) | −0.29 (−0.38;−0.19) | −0.07 (−0.18;0.04) | −0.04 (−0.14;0.07) | 0.03 (−0.08;0.14) |
| Testosterone | – | 0.84 (0.82;0.86) | 0.71 (0.67;0.74) | 0.71 (0.67;0.74) | −0.07 (−0.14;0.00) | 0.36 (0.29;0.43) | 0.20 (0.12;0.28) | 0.23 (0.15;0.30) |
| Free testosterone | – | – | 0.64 (0.60;0.68) | 0.70 (0.66;0.73) | −0.59 (−0.64;−0.54) | 0.27 (0.20;0.35) | 0.09 (0.01;0.17) | 0.24 (0.16;0.31) |
| Androstenedione | – | – | – | 0.54 (0.49;0.59) | −0.14 (−0.21;−0.07) | 0.35 (0.27;0.42) | 0.30 (0.22;0.37) | 0.33 (0.25;0.40) |
| DHEAS | – | – | – | – | −0.24 (−0.30;−0.17) | 0.23 (0.16;0.31) | 0.00 (−0.08;0.08) | 0.05 (−0.03;0.13) |
| SHBG | – | – | – | – | – | 0.02 (−0.06;0.10) | 0.15 (0.07;0.23) | −0.08 (−0.16;0.00) |
| Estrone[b] | – | – | – | – | – | – | 0.74 (0.70;0.77) | 0.73 (0.69;0.77) |
| Estradiol[b] | – | – | – | – | – | – | – | 0.97 (0.97;0.98) |
| *Postmenopausal women* | | | | | | | | |
| C-peptide | 0.04 (−0.06;0.13) | 0.19 (0.10;0.29) | 0.12 (0.02;0.21) | 0.01 (−0.09;0.11) | −0.42 (−0.50;−0.33) | 0.06 (−0.04;0.16) | 0.11 (0.01;0.20) | 0.23 (0.14;0.32) |
| Testosterone | – | 0.91 (0.90;0.92) | 0.67 (0.64;0.70) | 0.69 (0.66;0.72) | −0.07 (−0.13;−0.01) | 0.46 (0.42;0.51) | 0.47 (0.42;0.51) | 0.45 (0.40;0.49) |
| Free testosterone | – | – | 0.63 (0.60;0.67) | 0.67 (0.64;0.70) | −0.45 (−0.49;−0.40) | 0.47 (0.43;0.52) | 0.47 (0.43;0.51) | 0.57 (0.53;0.61) |
| Androstene-dione | – | – | – | 0.62 (0.59;0.66) | −0.09 (−0.15;−0.04) | 0.41 (0.37;0.46) | 0.44 (0.39;0.48) | 0.43 (0.38;0.48) |
| DHEAS | – | – | – | – | −0.16 (−0.22;−0.11) | 0.49 (0.45;0.53) | 0.46 (0.42;0.51) | 0.48 (0.43;0.52) |
| SHBG | – | – | – | – | – | −0.16 (−0.22;−0.10) | −0.12 (−0.18;−0.07) | −0.38 (−0.43;−0.33) |
| Estrone | – | – | – | – | – | – | 0.65 (0.62;0.68) | 0.65 (0.62;0.69) |
| Estradiol | – | – | – | – | – | – | – | 0.96 (0.95;0.96) |

Adjusted for: [a] age, batch and BMI.
[b] phase of menstrual cycle.

women) and was similarly, though more weakly, associated with serum levels of C-peptide ($r = 0.12$ and $r = 0.23$), androgens (range from 0.03 to 0.13 in premenopausal women and from 0.06 to 0.08 in postmenopausal women), and SHBG ($r = -0.26$ and $r = -0.23$) and somewhat stronger correlations were observed of IGFBP-3 with estrone ($r = -0.09$ and $r = 0.13$) and estradiol ($r = -0.13$ and $r = 0.09$).

## Discussion

In this large cross-sectional study, clear associations between excess body weight and serum levels of C-peptide, SHBG and free testosterone were observed among all women, and between body weight and total and free estrogens in postmenopausal women. Furthermore, C-peptide and IGF-I correlated independently with levels of sex-steroid hormones and SHBG. The relationships of adiposity with C-peptide, SHBG and free sex-steroid hormones in our study confirm results of previous studies [12, 18, 20–25]. High levels of insulin, for which C-peptide is a marker, have been associated with excess weight and inhibit SHBG production in the liver [7, 26]. As a consequence, the fraction of unbound sex-steroid hormones increases with increasing adiposity.

The design of this study needs some consideration. A cross-sectional study like this does not allow us to draw

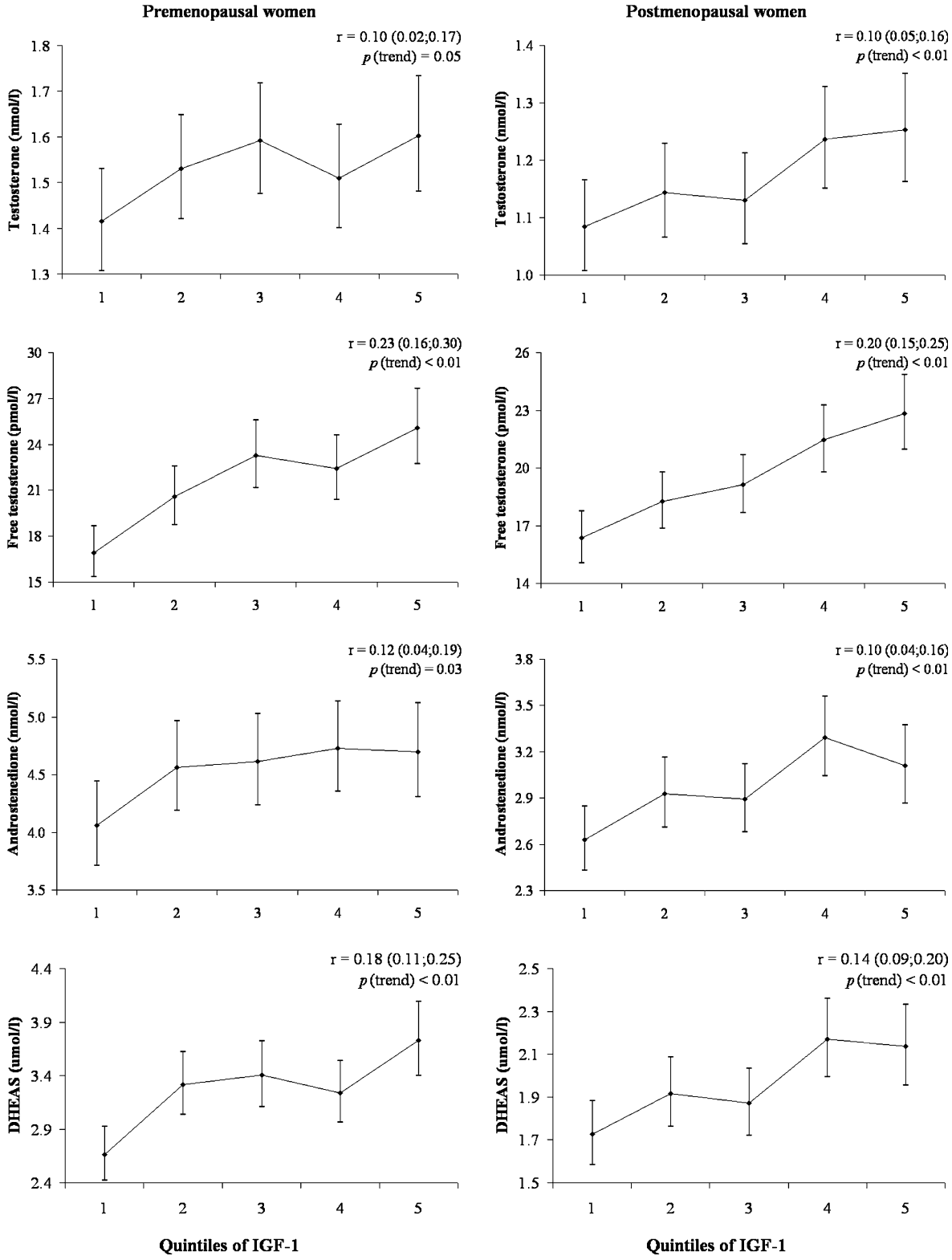


*Fig. 1.* Androgen concentrations across quintiles of IGF-I, adjusted for age, batch and BMI.

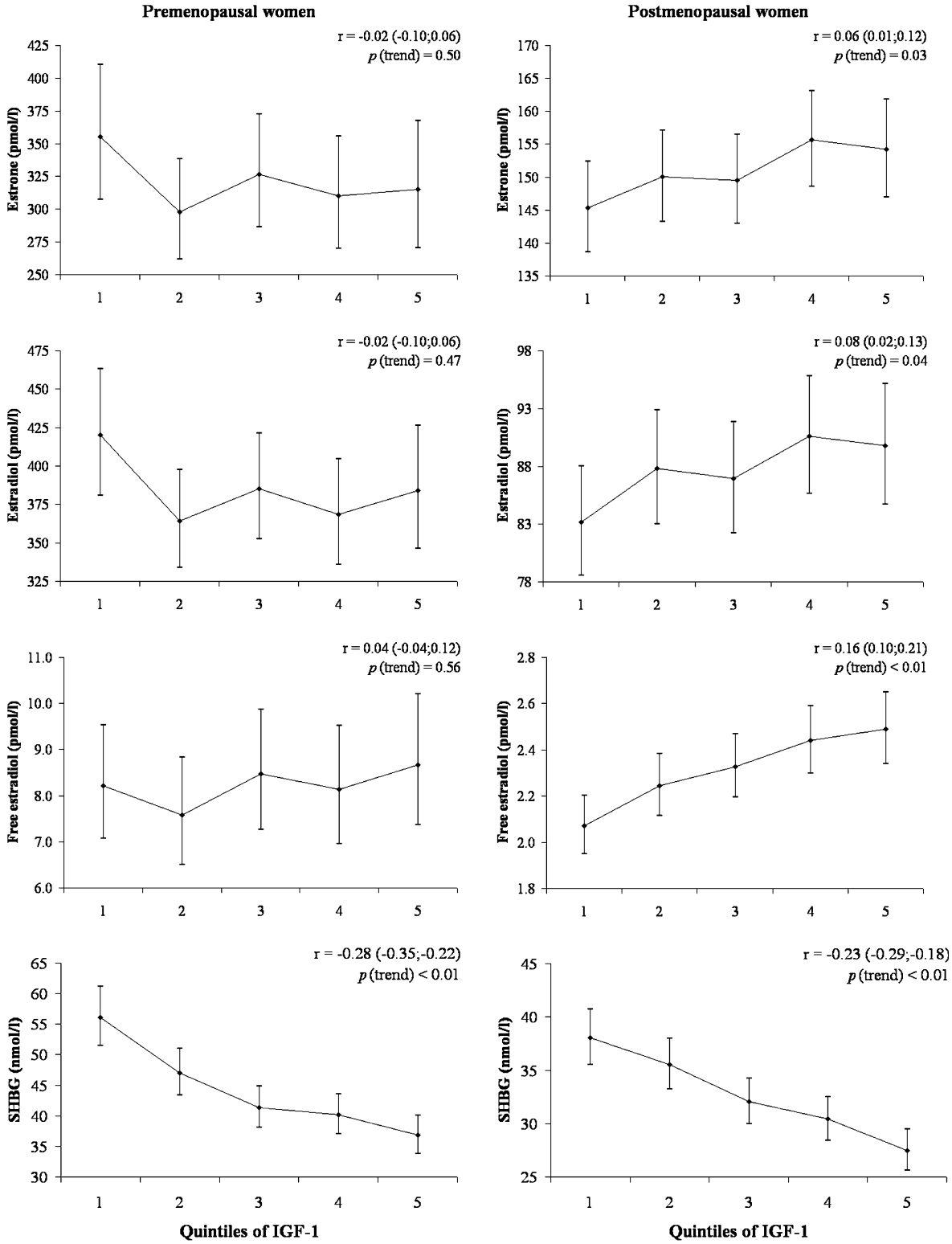


*Fig. 2.* Concentrations of estrogens[a] and SHBG across quintiles of IGF-I, adjusted for age, batch and BMI.
[a]Adjusted for phase of the menstrual cycle in premenopausal women.

conclusions about causal relationships, solely on the basis of the presented results. However, they do provide information about interrelationships of hormones, and together with results from *in vitro*, animal and clinical studies enable us to form hypotheses about potentially causal relationships.

The subjects in this study are controls from a nested case-control study. Controls were matched to cases on several factors, so they do not represent a random sample from a general population. This may influence the distribution of subject characteristics, but is not believed to change the direction of the observed correlations. It cannot be ruled out, however, that there is a quantitative effect, in that the presented correlations are somewhat stronger or weaker than would have been observed in a random sample.

The blood samples used in this study were obtained at a single point in time; hence we have to assume that the measured hormone levels are a good estimate of the mean hormone concentration over time within an individual. This was validated by reproducibility studies in other cohorts [27, 28] and supported by the absence of unexpected observations with regard to hormone levels or correlations between variables in our study. C-peptide was measured as a marker for insulin production. C-peptide had a longer half-life than insulin and is secreted in equimolar amounts. Therefore, C-peptide measurements reflect the long-term level of insulin more reliably than the level of insulin itself. In relation to serum levels of IGF-I and steroid hormones, as well as to body weight and cancer risk, these long-term effects are of interest.

### *BMI, waist circumference and sex-steroid hormone concentrations*

Body fat distribution is an important determinant of insulin resistance and hyperinsulinemia, which explains the somewhat stronger associations of waist circumference with insulin and the other hormones, as compared to BMI [6]. For total androgen measurements, a positive correlation of BMI and waist circumference with DHEAS was observed in premenopausal women, and a very weak correlation between adiposity and testosterone in all women. In previous studies, positive relationships between BMI and androgens were mainly found in women with polycystic ovary syndrome (PCOS) [29, 30], a syndrome characterized by hyperandrogenism, anovulation and a polycystic ovarian morphology [31, 32]. In healthy premenopausal women, the relationships between anthropometric indices of adiposity and total androgen levels are less clear. Either inverse [30, 33] or no [12, 21, 34] associations were found between BMI and androstenedione or DHEAS, and testosterone was either positively [33, 35, 36] or not [12, 34, 37] related to BMI. In postmenopausal women, a recent pooled analysis [38], as well as two other recent studies [12, 23], found a positive association of adiposity with testosterone and a weak positive association with androstenedione. Thus, previous studies as well as the present data indicate that adiposity and circulating androgens may be slightly positively related, but little evidence exists for a strong association in healthy women.

For total estrogen measurements, BMI and waist circumference were directly correlated with estrone and estradiol only in postmenopausal women, as observed in previous studies [12, 22, 23, 39]. In premenopausal women, results of former studies have been somewhat contradictory, but in general no association was observed between BMI and estrogens [20, 21, 33, 40], as in our study. Estrogens are mainly synthesized in the ovary in premenopausal women. At menopause, the ovaries cease producing estrogens and adipose tissue becomes the main site of estrogen production, by converting androgenic precursors from the ovaries and adrenal gland. The absence of an association between adiposity and both total and free estrogens in premenopausal women is probably due to the dominance of ovarian estrogen production before menopause, which is under tight control of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and regulated by feedback mechanisms [5]. In contrast, estrogen synthesis in adipose tissue is unregulated and directly correlated with the amount of adipose tissue, as observed in the direct association between adiposity and estrogen levels in postmenopausal women. Because these estrogens are converted from androgenic precursors, this may also explain why, in our study, androgens and estrogens were more strongly related in postmenopausal women than in premenopausal women.

### *Insulin and IGF-I: correlations with sex-steroid hormone concentrations*

Insulin and IGF-I are common to each other in many ways. Both peptides activate growth-promoting signal transduction pathways by interacting with their receptors, and, to some extent, can also bind to their mutual receptors or to hybrid insulin/IGF-I receptors [9, 10]. Other common effects include stimulation of sex steroid synthesis [41–45] and inhibition of SHBG production [24, 26, 46]. The associations of C-peptide and IGF-I with sex-steroid hormones and SHBG appeared to be independent, as the correlation between the peptides was low and mutual adjustment did not change the relationships

with sex-steroid hormones. The relationships between IGF-I and sex steroids were also independent of adiposity. Not surprising, since the relationship between IGF-I and BMI is nonlinear, as observed in our study and described in more detail previously [12, 18, 19].

C-peptide and IGF-I were both inversely correlated with SHBG and, as a result, positively with free sex steroids in postmenopausal women, as observed previously [13, 14, 20, 24]. In premenopausal women, IGF-I and free testosterone were also related. The lack of associations of C-peptide and IGF-I with free estradiol before menopause is, like for adiposity, probably due to regulation of ovarian sex steroid synthesis *via* LH and FSH. The positive relationship between IGF-I and free testosterone may be a result of increased androgen levels associated with IGF-I in both pre- and postmenopausal women, as discussed below. Thus, both insulin and IGF-I increase free sex steroids in postmenopausal women, mostly by inhibiting SHBG production. In premenopausal women these effects are less clear.

All androgens were positively correlated with IGF-I, as observed in some [12, 15, 47] but not all [13, 14] previous studies. C-peptide was significantly related only to androstenedione in postmenopausal women. An association between the other androgens and C-peptide may have been missed because of the small sample size of fasting women, for whom C-peptide measurements were available, or may not exist at all. Insulin stimulates androgen synthesis *in vitro*, and direct relationships between insulin and circulating androgens were reported in PCOS patients [29]. However [32, 36], previous cross-sectional studies have not shown any clear association between insulin or C-peptide and androgens among normo-androgenic women, without PCOS. Since the prevalence of PCOS is estimated to be 4% to 8% in Western European women [32], it is possible that the presence of hyperandrogenic women in our sample accounts for the slightly positive correlations between C-peptide and androgens observed in our study. The contradictory results for the associations of insulin or C-peptide with androgens correspond to those found for adiposity and circulating androgens, suggesting that insulin might have a role in the link between adiposity and androgen levels. However, this link might be restricted to, or at least be stronger in women with PCOS, compared to normo-androgenic women.

In the current study, total estrogens were very weakly correlated with IGF-I in postmenopausal women. Most former studies found no association between IGF-I and estrogens [12–14, 47]. The positive association between C-peptide and total estrogens before, but not after, adjustment for BMI supports the view that elevated estrogen levels in heavier postmenopausal women are mostly a direct consequence of the amount of adipose tissue, not of stimulation by insulin [41].

Besides the effects of insulin and IGF-I on sex steroid production observed *in vitro*, sex steroids, in turn, have shown to influence insulin and IGF-I levels. In animal models and clinical studies, androgen and estrogen treatments maintained normal insulin sensitivity at physiological concentrations but caused insulin resistance when administered in high doses [48]. Similarly, increased levels of circulating IGF-I were observed after androgen treatment in prepubertal boys [49] and estrogens have also shown to influence the GH/IGF-I axis [50], depending on the administration route. Increases in either hormone can thus lead to a vicious circle of elevated hormone levels [48]. However, the effects of insulin and IGF-I on sex steroids are believed to be of greater importance than those of sex steroids on these peptide hormones [14, 32, 48], and insulin and IGF-I have an additional effect on the bioavailability of sex steroids by decreasing SHBG levels.

In conclusion, these data suggest similar but independent relationships of insulin and IGF-I with levels of sex-steroid hormones. The observed relationships were similar in pre- and postmenopausal women, but the magnitude of the associations differed and generally stronger correlations were observed after menopause. Findings from this study support the idea that excess body weight increases sex-steroid hormone levels directly by increasing the capacity for estrogen production in adipose tissue, and increases levels of free sex steroids indirectly via effects on insulin and SHBG. Independent of adiposity and insulin, IGF-I is associated with increased androgen and possibly estrogen levels, and with increases in free sex steroid levels by inhibiting SHBG production.

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