

CLINICAL STUDY

Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women

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Abstract

Objective: Excess weight has been associated with increased risk of cancer at several organ sites. In part, this effect may be modulated through alterations in the metabolism of sex steroids and IGF-I related peptides. The objectives of the study were to examine the association of body mass index (BMI) with circulating androgens (testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEAS)), estrogens (estrone and estradiol), sex hormone-binding globulin (SHBG), IGF-I and IGF-binding protein (IGFBP)-3, and the relationship between sex steroids, IGF-I and IGFBP-3.

Design and methods: A cross-sectional analysis was performed using hormonal and questionnaire data of 620 healthy women (177 pre- and 443 post-menopausal). The laboratory measurements of the hormones of interest were available from two previous case-control studies on endogenous hormones and cancer risk.

Results: In the pre-menopausal group, BMI was not related to androgens and IGF-I. In the post-menopausal group, estrogens, testosterone and androstenedione increased with increasing BMI. The association with IGF-I was non-linear, with the highest mean concentrations observed in women with BMI between 24 and 25. In both pre- and post-menopausal subjects, IGFBP-3 did not vary across BMI categories and SHBG decreased with increasing BMI. As for the correlations between peptide and steroid hormones, in the post-menopausal group, IGF-I was positively related to androgens, inversely correlated with SHBG, and not correlated with estrogens. In the pre-menopausal group, similar but weaker correlations between IGF-I and androgens were observed.

Conclusions: These observations offer evidence that obesity may influence the levels of endogenous sex-steroid and IGF-related hormones in the circulation, especially after menopause. Circulating IGF-I, androgens and SHBG appear to be related to each other in post-menopausal women.

European Journal of Endocrinology 150 161–171

Introduction

Increased body weight and obesity have been implicated in the development of several common cancers, including those of the colon, the endometrium and the post-menopausal breast (1, 2). Excessive body weight may influence cancer risk by modulating the synthesis and metabolism of endogenous hormones, such as sex steroids, insulin, insulin-like growth factor-I (IGF-I) and their carrier proteins in the circulation (1–3).

Several prospective studies have now demonstrated that elevated circulating androgens, estrogens, insulin or IGF-I are directly related to increased risk of several cancers, including breast, colorectal, endometrial and ovarian cancers (1, 2, 4–6).

The metabolic effects of elevated body mass index (BMI), the measure of body adiposity most frequently used in epidemiology, vary according to gender and menopausal status (1, 3). Furthermore, research evidence suggests that the activity of the IGF system

and several sex-steroid hormones may be closely linked, but only a few large studies have reported on the cross-sectional relationships between peptide and sex-steroid hormones in healthy women (7–9). Better knowledge of the effect of excess body weight on hormone metabolism and of the associations between endogenous hormones is needed to improve our understanding of the mechanisms relating BMI and hormone levels to cancer risk.

The objectives of the current study were to examine the association of BMI with circulating androgens (testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEAS)), estrogens (estrone and estradiol), sex hormone-binding globulin (SHBG), IGF-I and IGF-binding protein (IGFBP)-3, and the relationship between sex steroids, IGF-I and IGFBP-3. We used the hormonal data obtained from 620 healthy women who had been selected as control subjects in previous case-control studies on ovarian and endometrial cancer (5, 6).

Materials and methods

Study population

Six hundred and twenty women who served as control subjects in previous nested case-control studies on endogenous hormone levels and ovarian ($n = 308$) (6) or endometrial cancer ($n = 312$) (5) were included in the current study. The original case-control studies were nested within three collaborating prospective cohorts – the New York Women's Health Study (NYUWHS), the Northern Sweden Health and Disease Study (NSHDS) and the Study of Hormones and Diet in the Etiology of Breast Cancer (ORDET) – and included only women who were not using exogenous hormones at the time of blood donation. The majority of women (53%) included in the current study were from the NYUWHS, 37% were from the NSHDS and 10% from the ORDET cohort. The age range at blood donation of the women included in the study was only slightly narrower than the age range of all the women recruited in the three parent cohort studies.

At recruitment, measurements of body height and weight were taken for all participants of the ORDET study and for 66% of the NSHDS participants. In the NYUWHS cohort and the remaining NSHDS participants, height and weight at baseline were self-reported. Analysis in a sub-sample of the NSHDS (as part of a study on breast cancer (10)), showed a very high correlation of self-reported with measured height ($r = 0.97$) and a less strong correlation with weight (0.75). The reliability of repeated (at intervals of 1 or more years) self-reported data for height and weight in the NYUWHS has been shown to be very high (intraclass correlations 0.98 for height and 0.95 for weight (11)).

At recruitment, all participants from the three cohort studies had given written consent for the use of their

blood samples in future research projects. The Ethical Review Boards of New York University School of Medicine, the University of Umeå, the Istituto Nazionale per lo Studio e la Cura dei Tumori in Milan and the International Agency for Research on Cancer in Lyon periodically reviewed and approved the research studies utilized in the present report.

Laboratory analyses

All hormonal measurements were performed at the Hormone Laboratory of the International Agency for Research on Cancer (IARC), Lyon, France, on either serum samples (NYUWHS cohort) or heparinized plasma samples (NSHDS and ORDET cohorts) from study subjects. Samples from three standard sera were inserted in each analytical laboratory batch to control the quality of the measurements.

Sex-steroid hormones were measured by the following assays: testosterone and DHEAS by radioimmunoassay (RIA) with reagents from Immunotech, Marseille, France; androstenedione, estradiol and estrone by double-antibody RIA with reagents from Diagnostic Systems Laboratories (Webster, TX, USA); and SHBG by immunoradiometric assays with reagents from Cis-bio, Gif-sur-Yvette, France. The mean intra-batch coefficients of variation were 8.2% for a testosterone concentration of 0.3 ng/ml, 6.3% for an androstenedione concentration of 0.5 ng/ml, 4.6% for a DHEAS concentration of 40.0 µg/dl, 3.5% for an estradiol concentration of 30 pg/ml, 5.3% for an estrone concentration of 20.0 pg/ml and 4.4% for an SHBG concentration of 40.0 nmol/l. The mean inter-batch coefficients of variations for the same concentrations were 10.9% for testosterone, 9.0% for androstenedione, 13.0% for DHEAS, 7.3% for estradiol, 14.3% for estrone and 9.0% for SHBG.

IGF-I and IGFBP-3 were measured by double-antibody immunoradiometric assays with reagents from Diagnostic Systems Laboratories. The protocol for IGF-I included an acid-ethanol extraction to release IGF-I from its binding proteins. The mean intra-batch coefficients of variation were 1.5% for an IGF-I concentration of 150 ng/ml and 4.8% for an IGFBP-3 concentration of 3800 ng/ml. The mean inter-batch coefficients of variation for the same concentrations were 3.4% for IGF-I and 4.2% for IGFBP-3.

All laboratory assays were performed between December 2000 and February 2002.

For the current study, 595 measurements of IGF-I, 585 of IGFBP-3, 482 of testosterone, 488 of androstenedione, 486 of DHEAS, 486 of SHBG, 310 of estrone and 125 of estradiol measurements were available. The difference in numbers of peptide and sex-steroid hormone measurements is due to the inclusion of measurements from the original case-control studies only if they were performed at the IARC Hormone Laboratory and because estrogens were measured in

samples of women who were post-menopausal at blood donation. The menopausal status of study subjects with inconclusive questionnaire data and who were 45–55 years old at recruitment was confirmed by follicle-stimulating hormone (FSH) measurement. Women were classified as post-menopausal if their FSH measurement was >12.75 IU/l. The FSH cut-off points were determined on the basis of questionnaire and age-distribution data for more than 300 women from the three cohorts.

Statistical analyses

All hormone data were log-transformed to reduce departures from the normal distribution. The statistical analyses were performed using the Statistical Analysis System software program (SAS Institute, Cary, NC, USA). Multivariate regression analyses were performed, using the SAS generalized linear model procedure, to estimate geometric mean hormone levels by subgroups of BMI, adjusted for age, cohort and case-control sub-study and to test for differences in mean hormone levels across categories of interest. Spearman partial correlations, similarly adjusted, were calculated. The effect of BMI on sex-steroid hormones, IGF-I and IGFBP-3 was studied across five BMI categories which were defined according to BMI distribution in pre- and post-menopausal women separately (BMI categories: ≤ 21.5 , 21.5–23.5, 23.5–25, 25–27 and >27 for pre-menopausal women; and ≤ 22.5 , 22.5–24, 24–25, 25–30 and >30 for post-menopausal women). Tests for linear trend were performed by scoring the categories according to the median BMI values and entering the dependent variable on a continuous scale. *P* values of <0.05 were considered statistically significant.

Free testosterone and estradiol concentrations were calculated on the basis of total testosterone,

total estradiol and SHBG measurements as described previously (12). A validation study conducted at the IARC Hormone Laboratory (where all hormone analyses were conducted) compared measurements of free testosterone and free estradiol concentrations obtained by dialysis plus an in-house RIA after extraction and chromatographic purification (reference method) with those calculated from total serum concentrations of testosterone or estradiol and SHBG, as measured by direct, commercial RIAs. The study indicated that theoretical calculations are valid for the determination of free testosterone and estradiol concentrations (12).

Results

Selected characteristics of the study population by menopausal status are presented in Table 1. At recruitment, 29% of the study subjects were pre-menopausal (177 women; mean age 44.3 years) and 71% were post-menopausal (443 women; mean age 57.6 years). Pre- and post-menopausal women had similar mean heights but post-menopausal women were slightly heavier and had higher BMI than pre-menopausal women.

Differences in mean hormone levels between pre- and post-menopausal women reached statistical significance for androstenedione, SHBG and IGFBP-3 (Table 1), but after adjustment for BMI only the difference in mean androstenedione levels remained significant (1.10 (0.96–1.25) in pre- versus 0.90 (0.83–0.99) in post-menopausal women, $P < 0.04$). Estrogen levels in pre- and post-menopausal women could not be compared, as estrogen measurements were available only for post-menopausal women.

Spearman correlations between steroid and peptide hormones, BMI and age (adjusted for cohort study, age at recruitment and case-control sub-study) in pre- and post-menopausal women are presented in Table 2.

Table 1 Geometric mean (5th–95th percentile) of BMI, SHBG and sex hormones by menopausal status, adjusted for cohort study, age at blood donation and case-control sub-study.

	Pre-menopausal women		Post-menopausal women		<i>P</i> value*
	<i>n</i>	Geometric mean (5–95%)	<i>n</i>	Geometric mean (5–95%)	
Age (years)	177	44.7 (36.9–50.9)	443	57.6 (49.3–66.6)	<0.0001
BMI	171	24.2 (20.0–30.2)	428	25.7 (20.4–33.9)	0.14
Weight (kg)	173	63.2 (49.0–85.5)	434	66.5 (51.5–90.8)	0.37
Height (cm)	172	162 (150–173)	432	161 (152–171)	0.60
Testosterone (ng/ml)	167	0.27 (0.10–0.68)	315	0.18 (0.04–0.68)	0.97
Free testosterone (pmol/l)	166	12.3 (4.1–36.0)	314	9.0 (1.4–38.2)	0.46
Androstenedione (ng/ml)	168	1.31 (0.56–2.54)	320	0.82 (0.29–2.34)	0.02
DHEAS (μ g/dl)	167	131.1 (47–303)	319	82.8 (31–274)	0.28
Estrone (pg/ml)	—	—	310	20.2 (11.1–44.7)	—
Estradiol (pg/ml)	—	—	125	23.2 (10.8–45.3)	—
Free estradiol (pmol/l)	—	—	125	2.10 (0.82–4.47)	—
SHBG (nmol/l)	167	50.1 (27.3–103)	319	42.5 (16.6–98.7)	0.02
IGF-I (ng/ml)	163	202.2 (99–359)	432	141.3 (64–290)	0.20
IGFBP-3 (ng/ml)	162	3615 (2312–4782)	423	3854 (2629–5449)	0.03

* General linear model.

Table 2 Spearman partial correlation coefficients between hormonal variables, adjusted for cohort, study and age at blood sampling. Hormone measurements were available as follows: 163 for IGF-I, 162 for IGFBP-3, 167 for testosterone, 166 for free testosterone, 168 for androstenedione, 167 for DHEAS and 167 for SHBG in pre-menopausal women and 432 for IGF-I, 423 for IGFBP-3, 315 for testosterone, 314 for free testosterone, 320 for androstenedione, 319 for DHEAS, 310 for estrone, 125 for total and free estradiol and 319 for SHBG for post-menopausal women.

	Testosterone	Free testosterone	DHEAS	Estrone	Estradiol	Free estradiol	SHBG	IGF-I	IGFBP-3	BMI	Age
Androstenedione											
Pre-menopausal	0.64‡	0.58‡	0.43‡	—	—	—	0.01	0.21*	0.09	−0.12	−0.24†
Post-menopausal	0.59‡	0.58‡	0.54‡	0.32‡	0.38‡	0.30†	−0.13*	0.16†	0.09	0.17†	−0.11*
Testosterone											
Pre-menopausal	—	0.90‡	0.68‡	—	—	—	0.02	0.14	0.08	0.00	−0.28†
Post-menopausal	—	0.92‡	0.61‡	0.39‡	0.36‡	0.33†	−0.11*	0.17†	0.04	0.17†	−0.08
Free testosterone											
Pre-menopausal	—	—	0.68‡	—	—	—	−0.37‡	0.14	0.07	0.05	−0.28†
Post-menopausal	—	—	0.61‡	0.48‡	0.36‡	0.46‡	−0.45‡	0.22†	0.14*	0.31‡	−0.09
DHEAS											
Pre-menopausal	—	—	—	—	—	—	−0.09	0.11	0.13	0.01	−0.20*
Post-menopausal	—	—	—	0.42‡	0.25†	0.24†	−0.20†	0.18†	0.06	0.10	−0.18†
Estrone											
Post-menopausal	—	—	—	—	0.50‡	0.55‡	−0.36‡	0.05	0.09	0.32‡	−0.16†
Estradiol											
Post-menopausal	—	—	—	—	—	0.90‡	−0.12	−0.11	−0.25†	0.22*	−0.43‡
Free estradiol											
Post-menopausal	—	—	—	—	—	—	−0.48‡	−0.05	−0.14	0.38‡	−0.42‡
SHBG											
Pre-menopausal	—	—	—	—	—	—	—	0.00	−0.08	−0.13	0.06
Post-menopausal	—	—	—	—	—	—	—	−0.17†	−0.29‡	−0.43‡	−0.02
IGF-I											
Pre-menopausal	—	—	—	—	—	—	—	—	0.30	−0.04	−0.32‡
Post-menopausal	—	—	—	—	—	—	—	—	0.47‡	−0.06	−0.19‡
IGFBP-3											
Pre-menopausal	—	—	—	—	—	—	—	—	—	−0.13	−0.02
Post-menopausal	—	—	—	—	—	—	—	—	—	0.03	−0.04

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.0001$.

Age was inversely correlated with all measured androgens in pre-menopausal women and with DHEAS, androstenedione and estrogens in post-menopausal women. In both pre- and post-menopausal women, age was inversely related to IGF-I, but not to IGFBP-3 or SHBG.

There were positive correlations between circulating concentrations of sex-steroid hormones in both pre- and post-menopausal women. In general, the estrogen-androgen correlations (range from 0.24 to 0.48) were weaker than androgen-androgen (range from 0.43 to 0.92) or estrogen-estrogen correlations (range from 0.50 to 0.90) (Table 2). Circulating levels of IGF-I correlated directly with levels of IGFBP-3 in pre- and post-menopausal women ($r = 0.30$ and 0.47 , $P < 0.0001$ respectively).

BMI did not correlate with androgen levels in pre-menopausal women, but direct associations with androgens and estrogens were observed among post-menopausal women. In both pre- and post-menopausal women, BMI was inversely associated with SHBG and there was no monotonic correlation with IGF-I or IGFBP-3 (Table 2). A similar pattern of the variation of hormone levels with increasing BMI was observed when the hormonal data were presented as geometric mean concentrations across BMI categories (Figs 1 and 2). The only exception was IGF-I concentrations in post-menopausal women, which showed a clear bell-shape curve, with peak IGF-I levels in women who had a BMI between 24 and 25 and lowest in women with BMI < 22.5 and BMI > 30.0 (heterogeneity < 0.01). The observed variation of IGF-I levels across categories of BMI was not influenced by adjustments for either any of the androgens in both pre- and post-menopausal women or for estrogens in post-menopausal women. The pattern of the associations between hormone levels and BMI did not differ in subgroups of subjects with measured or self-reported anthropometry data.

IGF-I correlated directly with all measured androgens. The associations were stronger, and statistically significant in post-menopausal (range 0.16–0.22; $P < 0.01$ for all correlations) than in pre-menopausal women (range 0.11–0.21). The associations between estrogens and IGF-I were very weak (range -0.11 – 0.05) and there was no significant variation of mean IGF-I levels across quintiles of estrogen concentrations, although mean IGF-I levels were somewhat lower in the last two quintiles of total estradiol. IGFBP-3 was inversely correlated to estradiol levels ($r = -0.25$; $P < 0.01$), but its correlation with estrone and free estradiol did not reach statistical significance (range -0.14 – 0.09). SHBG concentrations were inversely related to IGF-I and IGFBP-3 levels and declined across quintiles of IGF-I in post-, but not pre-menopausal women (Table 2).

As expected, there was an inverse correlation of SHBG with the calculated free testosterone and free estradiol (Table 2).

Discussion

The strength of this cross-sectional study is that it included large numbers of both pre- and post-menopausal women with measurements of circulating androgens, SHBG, IGF-I and IGFBP-3 and, for a proportion of the post-menopausal women, estrone and estradiol. A limitation of this study is that it was based on hormone measurements performed on blood specimens obtained at a single point in time. Thus, although the sex-steroid and peptide hormones of interest have been reported to have a good to high reproducibility (13–15), it is likely that the observed associations had been somewhat attenuated as a result of physiological within-subject fluctuations in hormone concentrations at any given point in time and variability related to laboratory error. An additional concern is the relatively narrow age range of women classified as pre-menopausal. Most (82%) of these subjects were aged 40 or older, which implies that a number of them were effectively perimenopausal at the time of blood sampling.

Effect of BMI on hormone levels

Among the best established metabolic effects of obesity on circulating endogenous hormones are the progressive reduction in SHBG with increasing BMI in both pre- and post-menopausal women and the direct association with estrogens in post-menopausal women (1, 3). Our data are consistent with these observations. A proposed mechanism for the reduction in SHBG is related to the concomitant rise in insulin levels with increasing BMI. Insulin has been shown to inhibit the hepatic synthesis of SHBG (1, 2). In post-menopausal women, estrogens are produced from the conversion of precursor androgens or other estrogens, mainly in the adipose tissue, and their production is not regulated by feed-back mechanisms (1, 16). As a consequence, after menopause, estrogen concentrations are directly related to the amount of adipose tissue. The increase in mean free estradiol concentrations across BMI categories observed in our data was greater than the increase in total estradiol concentrations (69 versus 29%), which is consistent with the dual effect of obesity toward increasing estrogen production and decreasing SHBG in the circulation.

In pre- and post-menopausal women, increased BMI, waist-hip ratio or abdominal obesity have been associated with either no change (17–21) or with an increase (3, 22–27) in total testosterone concentrations. Most of the studies in pre-menopausal women have shown an increase in free testosterone with increasing body weight (17, 22, 23, 28, 29), but very few such studies have been reported in post-menopausal women (20, 21). Many previous studies did not find an association of obesity with levels of androstenedione (18, 23, 28–31) or DHEAS (17, 21, 23, 26–28, 30) in either pre- or post-menopausal women.

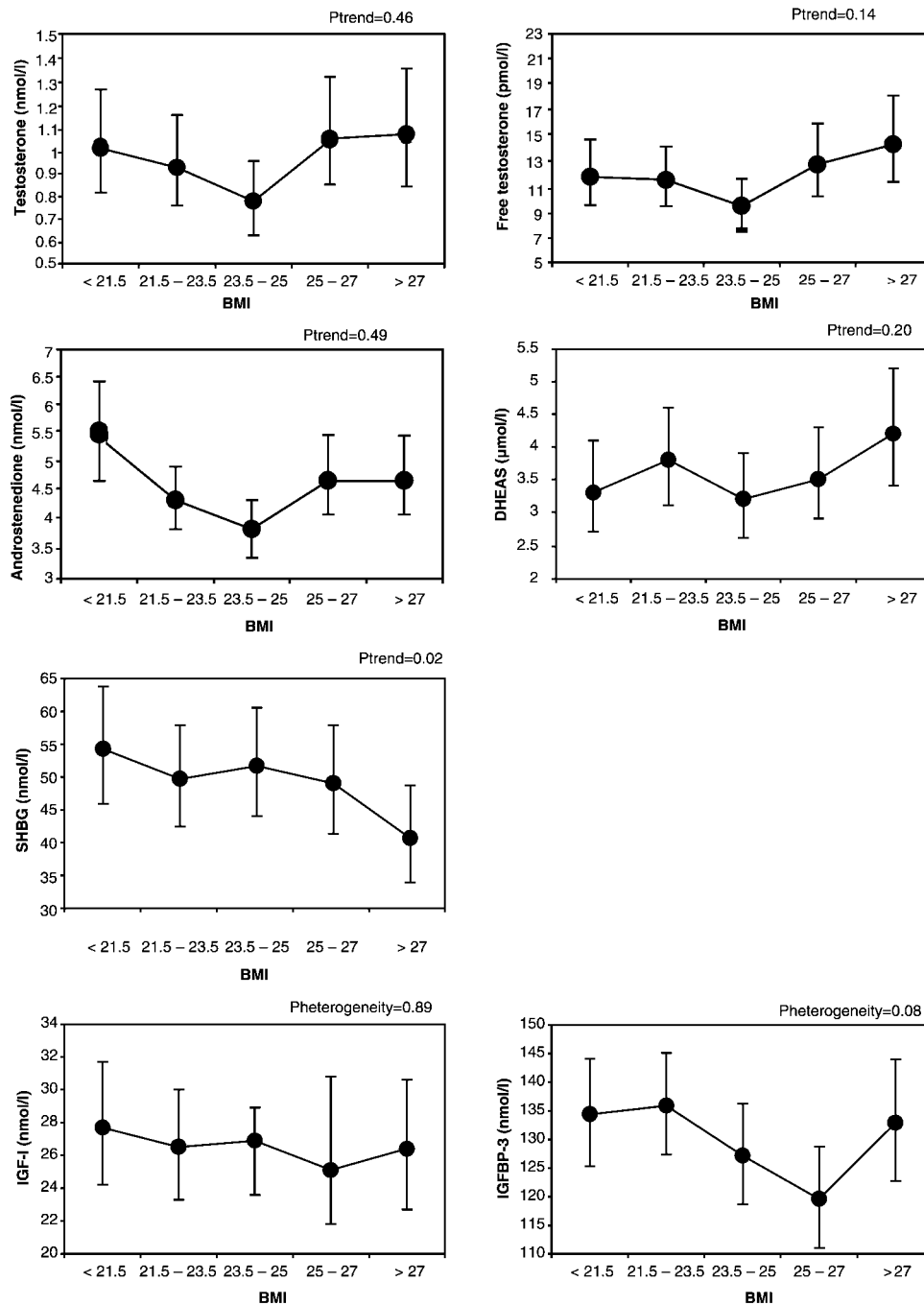


Figure 1 Variation of sex-steroid and peptide hormones across BMI categories among pre-menopausal women. *P* values for linear trend (Ptrend) were calculated by scoring the categories according to the median BMI values.

It has been proposed that the relationship between BMI and androgens is mediated by obesity-related changes in insulin and bioavailable IGF-I. *In vitro* studies have shown that both insulin and IGF-I can stimulate ovarian androgen synthesis (32-34). However, this 'gonadotropic' effect of insulin and IGF-I may be of less significance before menopause when circulating sex-steroid hormones are under the tight control of

luteinizing hormone (LH) and FSH and regulated by powerful feed-back mechanisms. Some studies have suggested that the adipose tissue, with its 17 β -hydroxysteroid dehydrogenase activity, may also be an important site of peripheral testosterone production (35). Finally, decreased SHBG concentrations, characteristic of obesity, would lead to an increase in the free testosterone fraction in both pre- and post-menopausal women (3).

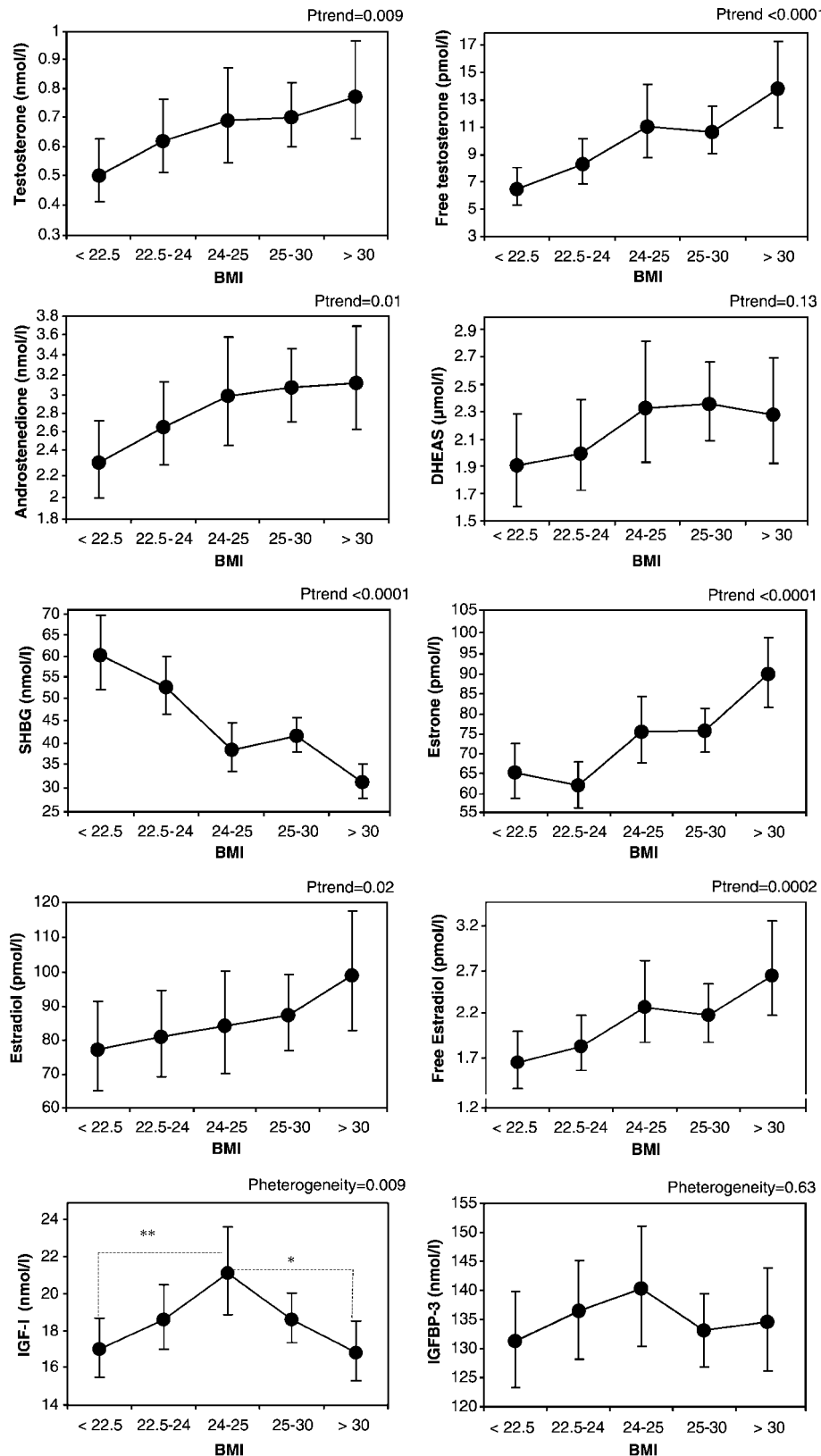


Figure 2 Variation of sex-steroid and peptide hormones across BMI categories among post-menopausal women. *P* values for linear trend (Ptrend) were calculated by scoring the categories according to the median BMI values. **P* < 0.05, ***P* = 0.05, difference in mean hormone levels between indicated categories.

In our study, no significant correlations between BMI and androgens were observed in pre-menopausal women, although there was a non-significant 25% increase in free testosterone in women with BMI >27 when compared with those with BMI <21.5. In post-menopausal women, mean levels of all androgens increased across BMI categories, but the increase in the top BMI category in comparison with the lowest was greatest for levels of free and total testosterone (153 and 65% respectively). The increase in DHEAS – an androgen synthesized exclusively by the adrenal glands – was much less pronounced and not significant. Thus, these results suggest that in post-menopausal women, increased BMI may lead to an enhanced ovarian synthesis of androgens.

Similarly to other studies (7, 15, 36, 37), only very weak monotonous correlations of BMI with IGF-I or IGFBP-3 were observed. In the post-menopausal group, there was a clear non-linear association of IGF-I with obesity, with the highest IGF-I levels in women with BMI between 24 and 25 kg/m². Similar findings have been reported previously (36, 38). The non-linear relationship of BMI with IGF-I may be the expression of obesity-related changes in the synthesis of insulin, growth hormone (GH), GH receptor, IGFBP-1 and IGFBP-2 (2, 38). In lean individuals, or after prolonged fasting, the low endogenous insulin production is associated with a decreased GH receptor levels, resulting in resistance of IGF-I synthesis in response to GH stimulation and a decrease in circulating IGF-I levels (2, 38). In obese subjects, elevated insulin levels increase the free fraction of IGF-I (through decreasing IGFBP-1 and IGFBP-2) with a consequent negative feed-back on the secretion of GH from the pituitary and a reduction in total circulating IGF-I (2).

In contrast to the results in post-menopausal women, mean IGF-I concentrations did not differ across BMI categories in the pre-menopausal group. At least partially, the lack of association in pre-menopausal women might be due to the narrower range of BMI and the small number of subjects with BMI ≥30 (*n* = 9). However, differences in the responsiveness of IGF-I to GH stimulation and in the proportion of lean and fat body mass could also contribute to the different pattern of the association of BMI with IGF-I in pre- and post-menopausal women.

Several studies have reported an increase in IGFBP-3 with obesity (36, 39, 40), but in most of the studies, including the current one, there was little variation in IGFBP-3 levels across BMI categories.

Inter-relationship between sex-steroid hormones, SHBG, IGF-I and IGFBP-3

Sex steroids and IGF-I-related hormones undergo parallel changes throughout life. There is a dramatic increase during puberty and then a substantial decrease with age. Still, the precise mechanisms of

the links and the possible interactions between these hormone systems remain largely unknown.

It has been proposed that endogenous sex-steroid hormones stimulate GH and IGF-I synthesis, as supported by observations of a minimal or no pubertal growth spurt in patients with hypogonadism, and that patients with both true precocious puberty and GH deficiency can exhibit a growth spurt indistinguishable from that of children with true precocious puberty and normal GH secretion (41). Additionally, both oral and trans-dermal exogenous androgens (42, 43) or androgenic progestins (44, 45) cause elevations in circulating IGF-I. The effect of exogenous estrogens depends on the route of administration. Oral formulations decrease IGF-I, most likely because of a first-pass effect on the liver of pharmacological doses of estrogens, while trans-dermal applications of estrogens do not seem to influence circulating IGF-I (46–50). Although not uniform, studies in pre-menopausal women have shown some degree of correlation between IGF-I and circulating estrogens (18, 51, 52) and androgens (18, 53–55), but no substantial variation in IGF-I throughout the menstrual cycle (56–58).

Alternatively, IGF-I has been shown to influence the synthesis of sex-steroid hormones. Patients with isolated GH deficiency or with Laron syndrome (GH resistance from a defect in the GH receptor) have delayed appearance of pubertal signs and slow and protracted puberty, even though ultimately reaching full sexual development (59). In Laron-syndrome patients treated with high concentrations of exogenous IGF-I, levels of sex-steroid hormones have been shown to increase and some female patients developed hyperandrogenism with oligo/amenorrhoea and acne (59, 60). There is also *in vitro* evidence that IGF-I enhances the LH-dependent ovarian (33, 61, 62) and adrenal (63, 64) androgen production and that IGF-I might affect gonadotropin production at the pituitary level (65, 66).

In this study, IGF-I concentrations were directly associated with those of all androgens, but the correlations were statistically significant only in post-menopausal women and for androstenedione in pre-menopausal women. Similar observations were reported by Helle *et al.* (8), but not in two other large cross-sectional studies in post-menopausal women (7, 9). IGFBP-3 correlated only weakly with all androgens studied in both pre- and post-menopausal women, as also observed by others (7–9). Although the cross-sectional nature of this study does not allow conclusions to be drawn on the causal relationship between the sex steroid and the IGF-I axes, it does provide evidence for a direct association of IGF-I with circulating androgens in post- and possibly in pre-menopausal women.

As observed in most previous studies (7–9), IGF-I and IGFBP-3 were not related to estrone and total estradiol concentrations in post-menopausal women. However, Janssen *et al.* (7) observed a direct correlation

between free estradiol and IGF-I or IGFBP-3 levels, while in our data weak inverse correlations were found. The observations of several epidemiological studies, taken together with the lack of substantial variation in IGF-I levels during trans-dermal estrogen replacement therapy, argue for a weak effect of circulating estrogens on plasma IGF-I concentrations in post-menopausal women.

Our findings of inverse associations of IGF-I or IGFBP-3 levels with SHBG in post-menopausal women concur with the results of other cross-sectional studies (7–9). It has been proposed that the underlying mechanism is the inhibition of hepatic SHBG synthesis by IGF-I, as shown *in vitro* (67, 68). An interesting observation is that the inverse association of IGFBP-3 with SHBG was stronger than that of IGF-I with SHBG in our data (also after adjustment for BMI) and in some other studies (7–9). No association of either IGF-I or IGFBP-3 with SHBG was observed in pre-menopausal women. However, currently, there is no evidence suggesting that the effect of IGF-I on the liver synthesis of SHBG differs according to menopausal status.

In conclusion, the results of this cross-sectional analysis provide evidence that an increase in BMI influences the circulating levels of sex-steroid hormones, in both pre- and post-menopausal women. The effect of BMI appears to be stronger after menopause, in the absence of the powerful feed-back mechanisms that control the synthesis of androgens and estrogens before menopause. Moreover, the effect of BMI may be more evident against the background of low hormone concentrations after the cessation of ovulatory activity. Additionally, a clear non-linear relationship between BMI and circulating IGF-I was observed in post-menopausal women. The study also offers further evidence of an inverse association between IGF-I and IGFBP-3 concentrations and SHBG and of a direct association of IGF-I with total and free circulating androgens in post-menopausal women. The identification of potentially modifiable life-style or hormonal determinants of IGF-I and sex-steroid hormone concentrations is important because of the increasing epidemiological evidence linking these hormones to cancer risk.

Acknowledgements

Ms Yelena Afanasyeva, Ms Lynne Quinones and Ms Daniela Masciangelo provided technical assistance in the NYUWHs, Ms Åsa Ågren and Mr Hubert Sjödin helped with the management of the Swedish Biobank database, Ms D Del Sette, Dr E Meneghini and Dr E Mugno helped with the ORDET database, Mr David Achaintre, Ms Josiane Bouzac and Ms Beatrice Vozar performed the laboratory analyses and Ms Jennie Dehedin helped in the manuscript preparation. The study was supported by Public Health Service grants R01 CA81188, R01 CA81200, R01 CA812212, R01

CA34588 and P30 CA16087 from the US National Cancer Institute and P30 ES00260 from the National Institute of Environmental Science. The NSHDS is sponsored by the Swedish Cancer Society and the ORDET cohort is sponsored by the Italian Association of Cancer Research.

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Received 15 July 2003

Accepted 10 November 2003