

Elevated levels of circulating insulin-like growth factor-I, IGF-binding globulin-3 and testosterone predict hormone-dependent breast cancer in postmenopausal women: A case-control study

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Abstract. There is increasing evidence that different types of breast cancers are related to distinct risk factors. We analyzed the risk of breast cancer with respect to circulating insulin-like growth factor (IGF)-I, IGF-binding protein (IGFBP)-3, 17 β -estradiol, estrone, testosterone, androstenedione and sex hormone-binding globulin (SHBG), taking into consideration the characteristics of the tumors. Plasma hormone levels of 102 postmenopausal patients with breast cancer detected by mammography screening, and 102 matched controls were analyzed in relation to the histological type, the status of the estrogen receptor (ER), the progesterone receptor (PR) and the HER2 in the tumors. Significant positive associations were revealed between the IGF-I concentration and the overall risk of breast cancer (OR=3.1, 95% CI: 1.5-6.2), ER+PR+ breast cancer (OR=2.4, 95% CI: 1.1-5.4) and ER+PR-breast cancer (OR=4.3, 95% CI: 1.2-14.3) when the highest and the lowest ranges of IGF-I were compared. Significant associations were also found between the highest and the lowest quartiles of testosterone, resulting in OR=4.1 (95% CI: 1.8-9.4) for the risks of breast cancer and OR=5.8 (95% CI: 2.1-16.2) of ER+PR+ breast cancer. A synergy was seen between IGF-I and testosterone levels. When both plasma IGF-I and testosterone were in the highest quartile ranges, an OR=26.4 (95% CI: 1.6-426.5, p=0.021) was computed for breast cancer overall. No significant synergistic effects could be demonstrated with other parameters. There were significant, 2.5-fold (95% CI: 1.2-5.6), and 16-fold (95% CI: 2.0-133.5) increases in the overall risks of breast cancer and of ER+PR-breast cancer, respectively, when the highest and the lowest

quartiles of IGFBP-3 were compared. No associations were found between any of the hormones and the risk of ER-PR-tumors. The increased prevalence of ER+ breast cancers in patients with higher levels of IGF-I, IGFBP-3 or testosterone implicate these hormones in the etiology of hormone-dependent breast cancer. Additional analyses specific for breast cancer subtypes may shed light on the value of hormone determinations for tailored chemoprevention.

Introduction

Human breast cancers display diverse genetic, histological and radiomorphological patterns, natural histories and responses to treatments. There is increasing evidence that different types of breast cancers are related to distinct risk factors (1,2).

The experimental and epidemiological findings consistently demonstrate the relationship between high levels of circulating insulin-like growth factor (IGF)-I and the risk of breast cancer (3-5). Most epidemiological studies have revealed a significant association between IGF-I and the risk of breast cancer only in premenopausal women (6-12), but in some studies a high IGF-I concentration was a marker of the risk of breast cancer in postmenopausal women (13), or of a higher risk in both premenopause and postmenopause (14-18). The circulating IGF-I level has been proposed as a surrogate biomarker for the risk of breast cancer in premenopausal women (19), and for identifying those who may benefit from treatment with fenretinide, which acts, at least in part, by reducing the plasma IGF-I level (20). Moreover, tamoxifen which reduces the risk of the hormone-dependent breast cancer, was shown to reduce the level of circulating IGF-I (21). The inhibition of the growth of various experimental IGF-I-dependent cancers, including breast cancers, by the antagonistic analogs of growth-hormone releasing hormone is exerted in part by the inhibition of the secretion of GH and the resulting decrease in the levels of hepatic IGF-I (22).

Positive associations between the risk of breast cancer and the estrogen and androgen levels have been suggested in postmenopausal women (23-25). Synergistic effects of IGF-I and IGF-binding protein (IGFBP)-3 with estrone and

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Table I. Characteristics (mean \pm SE) of healthy controls and cases with breast tumors.

Group	Age (years)	BMI	Age at menarche (years)	Age at menopause (years)	Parity	Age at first delivery (years)	Family history of breast cancer (%)
Controls (n=102)	57.8 \pm 0.5	28.6 \pm 0.5	13.7 \pm 0.1	48.0 \pm 0.5	1.8 \pm 0.1	21.8 \pm 0.7	6 (5.8)
Cases (n=102)	58.1 \pm 0.6	28.6 \pm 0.5	13.5 \pm 0.2	48.9 \pm 0.5	1.7 \pm 0.1	22.7 \pm 0.6	13 (12.7)

testosterone on the risk of breast cancer have been observed in premenopausal and postmenopausal Chinese women (9).

The incidence of estrogen receptor (ER)/progesterone receptor-positive (PR+) breast cancer may be halved, by hormonal manipulations which decrease the estrogenic effects in the breast but ER-negative (ER-) tumors are not prevented (1,26). The histological type of the breast cancer and the expressions of ER, PR and HER2 are widely used in clinical practice as markers of hormone dependence. Some findings tend to indicate that certain hormones play a role in the development of specific breast cancers (25,27,28). Positive associations were found between increased IGF-I and IGFBP-3 levels, and the risk of ER+ breast cancer in postmenopausal women in one case-cohort study, but when IGF-I and IGFBP-3 were mutually adjusted, a significant relation remained only with IGFBP-3 (27). Strong associations between the circulating sex hormone levels and the risk of ER+ breast cancer were demonstrated in postmenopausal women, in two case-control studies (25,28). However, in a similar prospective case-control study, such associations were lacking, and the conclusion was drawn that the ER status of breast tumors is independent of the concentrations of endogenous estrogens (29). Estrogens and androgens were most strongly associated with *in situ* cancers in one study (25), but such associations were absent in another (30). Although epidemiological studies do not support the role of hormonal factors in the evolution of HER2+ breast cancers (31,32), to our knowledge, the possibility of an association between circulating hormone levels and the HER2 status of breast cancers has not been examined in case-control studies.

Accordingly, we set out to analyze the risk of breast cancer in relation to IGF-I, IGFBP-3 and the sex hormones, taking into consideration the histology and the status of the hormone receptors and the HER2 of the tumors. We report the findings of a case-control study in postmenopausal women.

Materials and methods

Study population. Between February 2002 and April 2004, women attending the national mammography-screening program were invited to provide blood samples for the measurement of hormones as prospective markers of the risk of breast cancer (within the Hungarian mammography-screening program started in 2002, 45-65 year-old women are being invited for bilateral 2-view mammograms taken every 1-2 years). The study was approved by the Institutional Review Board of the University of Szeged. A total of 2027 women agreed to participate, and each gave her written

informed consent. Some individuals (<10) refused participation. Only women without diabetes, malignancy and who did not receive hormone replacement therapy (HRT) within the last 6 months were included in the study. Each participant underwent an in-person interview involving the use of a standard questionnaire. The information collected included demographic features, the menstrual and reproductive history, the duration of past hormone exposure, the individual medical history, and the family medical history. In addition, 10 ml of blood was collected in sterile heparinized vacutainers, and immediately chilled. The plasma was separated and then divided into 1-ml aliquots and stored at -70°C until hormone determinations. Finally, the routine mammography-screening procedure including physical examination by a technician was carried out.

The menopausal status was established at the time of blood collection. A woman was defined as premenopausal if she had at least one natural menstrual cycle within the last 12 months, and as postmenopausal otherwise. Bilateral oophorectomy indicated a postmenopausal status. In the event of hysterectomy, the menopausal status was determined by the concentrations of plasma 17 β -estradiol, FSH and LH. Only postmenopausal women were included in the analysis. The cases comprised the early-breast cancer patients (mostly with non-palpable tumors) diagnosed within the mammography-screening program. All patients were operated on at the Department of Surgery, University of Szeged. The control population consisted of the healthy women with no evidence of breast abnormalities. Two patients initially regarded as healthy controls developed breast cancer during the 26-month recruitment period of the study and were, therefore, included in the breast cancer group. Two other participants in the study population were diagnosed with non-breast malignancies after the recruitment, and consequently were excluded from the study. Information on the diagnosis, the stage of the disease and the tumor characteristics (histological type, and ER, PR and HER2 status determined by standard immunohistochemical analysis) was abstracted from the medical records. Samples with HER2 2+ or 3+ staining were classified as HER2+, and the others as HER2-. For each case with breast cancer or a high-risk proliferative breast lesion, one control was matched (33) by age, menopausal status, body mass index (BMI), age at menarche, age at menopause, age at first full-term delivery and parity, and the duration of past exposure to hormones.

Laboratory analyses. Radioimmunoassays (RIAs) or immuno-radiometric assays (IRMAs) were utilized to determine IGF-I,

Table II. Plasma hormone concentrations (mean \pm SE) by case or control status and the characteristics of the tumors.

	IGF-I (ng/ml)	IGFBP-3 ($\times 10^2$ ng/ml)	17 β -estradiol (pg/ml)	Estrone (pg/ml)	Androstenedione (ng/ml)	Testosterone (nmol/l)	SHBG (nmol/l)
Controls (n=102)	115.2 \pm 8.1	50.2 \pm 0.9	18.8 \pm 1.6	40.85 \pm 7.7	1.82 \pm 0.07	1.82 \pm 0.10	54.1 \pm 2.8
Breast cancer (n=102)	157.7 \pm 8.2	52.6 \pm 0.94	17.0 \pm 1.1	31.6 \pm 1.7	1.73 \pm 0.07	2.36 \pm 0.11	52.9 \pm 2.5
p-value	0.0003	0.0775	0.3752	0.2428	0.39	0.0005	0.7681
ER+PR+ tumors (n=64)	149.4 \pm 9.9	50.5 \pm 3.1	18.4 \pm 1.5	33.9 \pm 2.4	1.8 \pm 0.1	2.5 \pm 0.1	51.1 \pm 3.4
ER+PR- tumors (n=23)	165.3 \pm 18.7	57.5 \pm 2.0	13.7 \pm 0.8	28.6 \pm 2.5	1.5 \pm 0.1	2.4 \pm 0.3	54.6 \pm 3.9
ER-PR- tumors (n=10)	169.2 \pm 28.3	57.1 \pm 1.1	12.7 \pm 1.3	24.7 \pm 2.7	1.36 \pm 0.1	1.9 \pm 0.3	54.7 \pm 7.6
p-value	0.62	0.0026	0.09	0.17	0.09	0.32	0.80
HER2+ tumors (n=21)	171.1 \pm 18.7	56.56 \pm 2.1	14.3 \pm 1.1	27.9 \pm 2.7	1.7 \pm 0.1	2.4 \pm 0.2	50.0 \pm 4.3
HER2- tumors (n=68)	152.5 \pm 10.3	51.21 \pm 1.1	18.5 \pm 1.6	33.5 \pm 2.3	1.8 \pm 0.1	2.5 \pm 0.1	52.6 \pm 3.3
p-value	0.39	0.02	0.15	0.2	0.95	0.79	0.69
Invasive tumors (n=91)	155.8 \pm 8.7	52.3 \pm 1.0	17.1 \pm 1.2	32.7 \pm 1.8	1.8 \pm 0.1	2.4 \pm 0.1	53.6 \pm 2.7
Noninvasive tumors (n=11)	173.4 \pm 23.5	54.7 \pm 2.1	16.6 \pm 3.6	22.64 \pm 3.6	1.6 \pm 0.2	1.9 \pm 0.3	47.3 \pm 6.9
p-value	0.51	0.43	0.90	0.06	0.43	0.14	0.44

IGFBP-3, androstenedione, 17 β -estradiol, estrone (Diagnostic Systems Laboratories, Webster, TX, USA), testosterone and sex hormone-binding globulin (SHBG) (Institute of Isotopes, Budapest, Hungary) concentrations. The hormones were measured in duplicates. The intra- and inter-assay coefficients of variation, for these methods, respectively, were: 8.6% and 6.9% for IGF-I, 4.9% and 4.2% for IGFBP-3, 7.0% and 12.6% for testosterone, 7.6% and 12.6% for SHBG, 5.4% and 8.3% for 17 β -estradiol, 5.3% and 6.5% for estrone, and 2.2% and 12.9% for androstenedione. To eliminate any measurement difference caused by batch-to-batch variation between the controls and the cases, we analyzed the matched control and case samples with the same batch.

Statistical analyses. The relationships between the plasma IGF-I and IGFBP-3 levels together with the concentrations of sex hormones and the risk of breast cancer were investigated. All statistical analyses were performed with Stata statistical software, version 8.0. The Chi-square test, the Student's t-test and one-way ANOVA were used. Matched data sets were analyzed by using the multiple logistic regression method. In the logistic regression model, the IGF-I ranges <100.0, 100.0-140.0, 140.0-180.0 and >180.0 ng/ml were used to determine the risk of breast cancer. To calculate ORs in relation to other hormones, the plasma hormone levels were grouped using the method of Connor (34). The goodness-of-fit of significant logistic models was checked by using the Hosmer-Lemeshow method. A probability level of $p < 0.05$ was considered statistically significant.

Results

The baseline characteristics of the 102 breast cancer cases and the 102 controls were equally distributed (Table I). Pathological examination of the breast cancer cases after surgery revealed ductal cancer *in situ* in 11, and invasive cancer in

91 cases. Among the invasive cancers, there were 82 ductal, 7 lobular and 2 medullary cancers found. The status of the ER/PR and the HER2 of the tumors was known in 98 and 89 cases, respectively. Sixty-four tumors were ER+PR+, 23 were ER+PR-, and 10 were ER-PR- (Table II). One tumor was ER-PR+ but was excluded from the analysis during the stratification by the ER/PR status of the tumors. Twenty-one tumors were classified as HER2+, and 68 as HER2- (Table II).

The plasma IGF-I level was significantly higher ($p = 0.0003$) for the breast cancer cases (157.7 \pm 8.2 ng/ml) than for the controls (115.2 \pm 8.1 ng/ml) in the overall population study (Table II). The molar IGF-I/IGFBP-3 ratio was significantly higher for the breast cancer cases than for the controls (0.11 \pm 0.05 vs. 0.08 \pm 0.05, $p = 0.0004$). The levels of IGFBP-3, 17 β -estradiol, estrone, androstenedione and SHBG did not differ between the two groups. However, there was a significant difference between the breast cancer cases and the controls in mean plasma testosterone level (2.36 \pm 0.11 vs. 1.82 \pm 0.10, $p = 0.0005$) (Table II). The hormone levels in the cases of breast cancer stratified by the tumor ER/PR, HER2 or invasive vs. noninvasive status are also shown in Table II.

There were significant negative correlations between the BMI and IGF ($r = -0.24$, $p = 0.014$), and between the BMI and IGFBP-3 ($r = -0.21$, $p = 0.030$) in the control group, but not in the breast cancer group. Significant correlations were found between IGF-I and IGFBP-3 ($r = 0.434$, $p < 0.001$ and $r = 0.450$, $p < 0.001$ in the control and the breast cancer group, respectively), between IGF-I and SHBG ($r = -0.186$, $p = 0.06$ and $r = -0.205$, $p = 0.039$ in the controls and the breast cancer cases, respectively), and between the BMI and SHBG ($r = -0.355$, $p = 0.0003$ and $r = -0.305$, $p = 0.002$ in the controls and the breast cancer cases, respectively). No significant correlations were found between the plasma IGF-I and sex hormone levels, or between the age and any of the hormone levels, except for IGF-I. Likewise, no associations were found between the BMI and the levels of the sex hormones.

Table III. Quartile ranges for plasma hormone levels based on the literature data (IGF-I) or the frequency distribution of the controls.

IGF-I (ng/ml)	<100.0	100.0-140.0	140.0-180.0	>180.0
IGFBP-3 ($\times 10^2$ ng/ml)	<43.9	43.9-49.4	49.4-54.7	>54.7
Estradiol (pg/ml)	<11	11.0-14.2	14.2-17.9	>17.9
Estrone (pg/ml)	<19.5	19.5-23.6	23.6-35.0	>35.0
Androstenedione (ng/ml)	<1.3	1.3-1.8	1.8-2.2	>2.2
Testosterone (nmol/l)	<1.1	1.1-1.7	1.7-2.4	>2.4
SHBG (nmol/l)	<34.4	34.4-48.5	48.5-67.9	>67.9

We detected decreased plasma IGF-I concentrations in multiparous women, but the difference did not attain the level of statistical significance.

The hormone levels were analyzed in a logistic regression model. To compute ORs, IGF-I measurements were categorized into the following ranges: <100.0, 100.0-140.0, 140.0-180.0, and >180.0 ng/m; the other hormone levels were set into quartiles, using the frequency distribution of the controls (Table III). Significant positive associations were revealed between the IGF-I concentration and the overall risk

of breast cancer (OR=3.1, 95% CI: 1.5-6.2, $p_{\text{trend}}=0.002$), the risk of ER+PR+ breast cancer (OR=2.4, 95% CI: 1.1-5.4, $p_{\text{trend}}=0.025$) and the risk of ER+PR- breast tumors (OR=4.3, 95% CI: 1.2-14.3, $p_{\text{trend}}=0.035$), when the highest and the lowest ranges of IGF-I were compared (Table IV). Such an association was lacking for the ER-PR- tumors. There were significant, 2.5-fold (95% CI: 1.2-5.6, $p=0.036$) and 16.3-fold (95% CI: 2.0-133.5, $p_{\text{trend}}=0.003$) increases in the risk of overall breast cancer, and in that of ER+PR- breast cancer, respectively, when the highest and the lowest quartiles of IGFBP-3 were compared (Table IV).

When the effect of testosterone was analyzed, significant associations were found as follows: OR=4.1 (95% CI: 1.8-9.4, $p=0.001$) and OR=5.8 (95% CI: 2.1-16.2, $p<0.001$) for the risk of overall breast cancer and for that of ER+PR+ breast cancer, respectively (Table IV). No significant associations were revealed by the analysis on stratification by the HER2 status or the histological classification of the tumors (data not shown).

When the associations between the plasma hormone levels and the risk of invasive vs. noninvasive breast cancer were calculated, OR=4.8 (95% CI: 1.1-20.8, $p=0.040$) and OR=2.9 (95% CI: 1.4-6.0, $p=0.004$), respectively were found, when the highest range of IGF-I was compared with the lowest. When testosterone was placed in the same model, OR=4.7 (95% CI: 2.0-11.3, $p=0.001$) was calculated for the risk of invasive breast cancer (Table IV), whereas no significant relationship was detected for *in situ* cancer.

The joint effects of the plasma levels of IGF-I, IGFBP-3 and testosterone were studied by including them in a multiple logistic regression model; for the risk of overall breast cancer, IGFBP-3 then lost its significant effect. However, IGFBP-3

Table IV. Overall risk of breast cancer according to quartile ranges of plasma hormone levels, and by the characteristics of the tumors.

	Number of controls/cases				OR/quartile ranges				95% CI		OR for trend	95% CI		p-value for trend
	1	2	3	4	1	2	3	4	1	2		1	2	
IGF-I ng/ml	50/28	1.0	17/20	2.1	0.95-4.65	14/18	2.3	0.99-5.30	21/36	3.1	1.50-6.22	1.43	1.14-1.80	0.002
ER+PR+	50/19	1.0	17/13	1.0	0.82-4.92	14/13	2.4	0.97-6.14	21/19	2.4	1.05-5.37	1.34	1.03-1.74	0.025
ER+PR-	50/5	1.0	17/6	3.5	0.95-13.0	14/2	1.4	0.24-8.16	21/9	4.3	1.2-14.3	1.49	1.02-2.16	0.035
Invasive	50/25	1.0	17/19	2.2	0.99-5.03	14/17	2.4	1.03-5.70	21/30	2.9	1.37-5.96	1.41	1.11-1.78	0.004
Noninvasive	50/3	1.0	17/1	0.98	0.09-10.06	14/1	1.2	0.11-12.35	21/6	4.8	1.1-20.8	1.71	1.02-2.86	0.040
IGFBP-3 ng/ml	26/12	1.0	23/20	1.7	0.74-4.03	27/21	1.5	0.66-3.29	26/49	2.5	1.15-5.60	1.30	1.01-1.67	0.036
ER+PR-	28/1	1.0	21/5	6.6	0.72-61.40	29/2	1.9	0.16-22.51	24/14	16.3	1.99-133.5	2.11	1.27-3.49	0.003
Estrone pg/ml	28/24	1.0	23/13	0.7	0.27-1.57	26/32	1.4	0.67-3.0	25/33	1.5	0.72-3.27	1.21	0.95-1.54	0.114
ER+PR+	28/14	1.0	23/7	0.6	0.21-1.76	26/18	1.4	0.57-3.33	25/25	2.0	0.86-4.66	1.32	1.00-1.75	0.045
Invasive	28/18	1.0	23/13	0.9	0.36-2.16	26/29	1.7	0.78-3.83	25/31	1.9	0.87-4.26	1.29	1.00-1.67	0.044
Testosterone nmol/l	26/12	1.0	23/20	1.9	0.75-4.67	27/21	1.7	0.69-4.10	26/49	4.1	1.77-9.39	1.53	1.18-1.98	0.001
ER+PR+	26/6	1.0	23/10	1.9	0.59-5.99	27/13	2.1	0.69-6.3	26/35	5.8	2.1-16.2	1.78	1.30-2.42	<10 ⁻³
c-erbB-2+	36/2	1.0	41/2	0.9	0.11-6.55	43/5	2.1	0.38-11.44	63/12	3.4	0.72-16.18	1.64	1.02-2.63	0.038
c-erbB-2-	30/8	1.0	31/12	1.5	0.52-4.04	34/14	1.5	1.56-4.18	41/34	3.1	1.26-7.66	1.45	1.10-1.91	0.007
Invasive	26/10	1.0	23/18	2.0	0.78-5.28	27/16	1.5	0.59-4.00	26/47	4.7	1.96-11.25	1.59	1.22-2.08	0.001

Table V. OR and 95% CI for postmenopausal breast cancers by the characteristics of the tumors.

Breast cancer	OR	95% CI	p (test for trend)
All			
IGF-I	1.37	1.06-1.76	0.016
IGFBP-3	1.13	0.85-1.49	0.395
Testosterone	1.52	1.17-1.98	0.001
ER+PR+			
IGF-I	1.41	1.04-1.90	0.025
IGFBP-3	0.96	0.69-1.34	0.841
Testosterone	1.82	1.33-2.51	<0.001
ER+PR-			
IGF-I	1.18	0.77-1.80	0.446
IGFBP-3	1.99	1.13-3.48	0.016
Testosterone	1.44	0.93-2.25	0.101
ER-PR-			
IGF-I	1.28	0.72-2.29	0.386
IGFBP-3	1.73	0.82-3.67	0.148
Testosterone	1.34	0.72-2.51	0.350
HER2+			
IGF-I	1.12	0.75-1.70	0.563
IGFBP-3	1.45	0.87-2.40	0.150
Testosterone	1.63	1.01-2.62	0.044
HER2-			
IGF-I	1.19	0.92-1.56	0.176
IGFBP-3	0.98	0.73-1.32	0.931
Testosterone	1.44	1.09-1.90	0.009
Invasive			
IGF-I	1.37	1.04-1.78	0.020
IGFBP-3	1.08	0.81-1.44	0.584
Testosterone	1.59	1.21-2.09	0.001
Noninvasive			
IGF-I	1.51	0.86-2.66	0.147
IGFBP-3	1.57	0.77-3.18	0.209
Testosterone	1.20	0.65-2.21	0.559

remained significant for the ER+PR- subtype (Table V). Their joint effects were also analyzed at different combinations of their quartile ranges. When both plasma IGF-I and testosterone were in the highest quartile ranges, an OR=26.4 (95% CI: 1.6-426.5, $p=0.021$) was computed for overall breast cancer. No significant synergistic effects could be demonstrated with other parameters.

Although there were no significant differences in the plasma estrone levels among cases and controls or the different subgroups of the breast cancer patients, a significant trend (OR~1.3) with increasing estrone levels was found for the risks of both ER+PR+ and invasive breast cancers (Table IV). Our data did not indicate a significant association between the risk

of breast cancer in general, or of any of the specific subgroups, with elevated levels of circulating 17 β -estradiol, androstenedione or SHBG.

Discussion

We found a significant positive association between the plasma IGF-I and IGFBP-3 levels and the overall risk of breast cancer; likewise, a strong relationship was observed between these same parameters and the risk of ER+ breast cancers. Most of the earlier prospective studies (6-12) demonstrated a significant association between IGF-I and the risk of breast cancer exclusively in premenopausal women, or failed to detect such a relationship in postmenopausal women (35,36). In other investigations, a high IGF-I concentration was a marker for the risk of breast cancer, irrespective of the menopausal status (14-18), and one investigation revealed a high breast cancer risk parallel to an elevated IGF-I concentration in postmenopausal Afro-American women (13). These studies (13-18), similarly to ours, were case-control studies, which may provide an explanation for the discrepancy and for our results. It has been proposed that, in case-control studies, the presence of the malignant tumor itself might affect the IGF-I and IGFBP-3 concentrations, thereby reflecting the tumor marker status rather than a true risk assessment (6). Singer *et al* investigated the IGF-I level in early breast cancer patients, but did not find any correlation between IGF-I and the tumor diameter (14). The IGF-I level is decreased in advanced cancer, probably because of the inanition (4,37). On the other hand, IGF-I has been shown to enhance the survival, migration and growth of early melanoma cells, but not to exert such an effect on melanoma cells from advanced lesions (38). These concerns led us to study only early cancer cases, when the phenotype of the tumor probably reflects the role of the hormones better than in advanced stages. On the other hand, since the levels of circulating IGF-I and IGFBP-3 depend strongly on race, lifestyle and diet (37,39), we cannot exclude the possibility that racial or nutritional factors also played a role in our findings.

The results on IGFBP-3 in the various studies are not in agreement, some show an increased risk (16,27), others a decreased risk (12), and still others no difference in postmenopausal women (10,15,35,36). Gronbaek *et al* analyzed the concentrations of the members of the IGF system in relation to the risk of breast cancer, taking into consideration the ER status of the tumors (27). In that case-cohort study, an increased risk of ER+ breast cancer with elevation in the levels of IGFBP-3 and IGF-II in postmenopausal women was demonstrated. Although no association was observed between the risk of overall breast cancer and an elevated IGF-I level, IGF-I was significantly associated with the risk of ER+ breast cancer. This association disappeared after mutual adjustment for the concentrations of the other members of the IGF system (27). In support of these findings, we demonstrated significant associations between the level of IGF-I and the risk of both ER+PR+ and ER+PR- breast cancers. At the same time, an elevated IGFBP-3 level was strongly related to the occurrence of the ER+PR- subtype, but not to that of the ER+PR+ subtype. When the joint effect of IGF-I, IGFBP-3 and testosterone was analyzed, only IGFBP-3 remained significant for

the ER+PR- subtype, suggesting an independent effect for IGFBP-3. It may be speculated that, as the ER+PR- and ER+PR+ breast cancers exhibit different responses to hormonal therapies (40), they represent different disease entities, thereby requiring different preventive interventions. To achieve this, specific risk-predictors are needed. Additional hormone receptor subtype-specific studies might support such a role for IGFBP-3.

We found a significantly higher plasma testosterone concentration in the breast cancer cases than in the controls, and a strong association between the risk of breast cancer and the plasma testosterone level, but there was no association with androstenedione. Enhanced testosterone levels have been shown to increase the risk of breast cancer in most studies (23,25,41,42), including case-control studies with a design similar to ours (9). Moreover, we detected a significant association between an elevated testosterone concentration and the risk of ER+PR+ breast cancers. This is consistent with the finding in a recent prospective study of a positive relation between an elevated testosterone level and the risk of ER+ breast cancer (25). However, we could not reveal an association between an elevated level of testosterone and the risk of ER+PR- breast cancers. This suggests that testosterone, a strong predictor for ER+PR+ breast cancers, is related to breast cancer risk differently than IGF-I and IGFBP-3.

Endogenous estrogens have been found to be significant breast cancer risk factors in prospective studies (23-25,28), but the case-control studies displayed significant heterogeneity (24). Although our study did not demonstrate association between the levels of circulating estrogens and the risk of overall breast cancer, increased plasma estrone levels were related to the risk of ER+PR+ and invasive breast cancers. In the meta-analysis by The Endogenous Hormones and Breast Cancer Collaborative Group it was concluded that the postmenopausal risk of breast cancer was related to the levels of the sex hormones (23). In the same study, the relative risk also increased with increasing BMI, while the magnitude of the risk was substantially reduced by adjustment for the serum estrogen concentrations. In contrast, adjustment for the androgens had little effect on the excess risk (23). Our study revealed a significantly elevated testosterone level, but no significant difference in the levels of the estrogens as compared with those in the healthy controls. A possible explanation for this finding could be that the breast cancer patients and the controls were well-balanced for the BMI, which is a marker of postmenopausal estrogen production, while the circulating testosterone level is independent of the BMI. For a better understanding of our findings, we analyzed the estrogen levels in relation to some characteristics of the study individuals and the levels of other hormones determined. Nonetheless, no correlation was found between the levels of the estrogens and age, the BMI or other hormones.

In accordance with others (23,43), we conclude that the SHBG level is inversely correlated with the BMI. The SHBG level did not display any association with the risk of breast cancer among postmenopausal women. This finding was consistent with the results of a similarly designed population-based case-control study (9) and a prospective case-control study (25). Lukanova *et al* reported that the IGF-I concentration correlated positively with the concentrations of

the androgens, but not with those of the estrogens (43). We did not observe correlations between the levels of IGF-I and any of the sex hormones. These results are consistent with the data of the case-control study of Yu *et al* (9).

To our knowledge, our study is the first in which hormone levels were examined in relation to the HER2 status of breast cancers. Our analysis suggests that the plasma IGF-I, IGFBP-3, 17 β -estradiol, estrone, androstenedione and SHBG concentrations are not related to the HER2 status of the tumors. The finding that testosterone is significantly associated with the risk of both HER2+ and HER2- tumors may be explained by the similar distribution of ER+ tumors within the HER2+ and HER2- subgroups. In a case-control study, risk factors for breast cancer such as an early age at menarche, a higher waist-hip ratio, and a family history of breast or ovarian cancer were associated with an elevated risk of both HER2+ and HER2- breast cancers (34). In another study, a large cohort of breast cancer patients were studied for the association of the HER2 status of the tumors and the endocrine history of the patients, including parity, the age at menarche and the age at menopause. HER2+ tumors occurred significantly more frequently than expected in women with a higher number of deliveries, later menarche and earlier menopause, when the protective endocrine factors were taken into account (31). The conclusion that most endocrine risk factors do not operate through an HER2 overexpression in breast carcinogenesis is consistent with our findings.

In the analysis by Missmer *et al*, both estrogens and androgens tended to be associated most strongly with *in situ* tumors (25). In contrast, no such association was found in a similar study (30). In our analysis, an elevated IGF-I level was the strongest indicator of the risk of *in situ* disease.

The only investigation of the joint effect of the members of the IGF system and the sex hormones was that by Yu *et al* (9). Their case-control study compared the plasma levels of IGFs and sex hormones in Chinese premenopausal and postmenopausal women matched for age and menopause. A synergistic effect on the risk of breast cancer was proposed for IGF-I or IGFBP-3 with estrone or testosterone among both premenopausal and postmenopausal women (9). We also found a strong synergy between IGF-I and testosterone on breast cancer risk, but no such effect was revealed for IGFBP-3.

Our case-control study pointed to positive relations between the IGF-I, IGFBP-3 and testosterone levels and the risks of both overall breast cancer and hormone-dependent breast cancer. The increased prevalence of ER+ breast cancers in patients with higher levels of IGF-I, IGFBP-3, testosterone and estrone implicate these factors in the evolution of hormone-dependent breast cancer. Additional breast cancer subtype-specific analyses may shed light on the value of hormone determinations for tailored chemoprevention.

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