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CIRCULATING LEVELS OF INSULIN-LIKE GROWTH FACTOR I, ITS BINDING PROTEINS -1,-2, -3, C-PEPTIDE AND RISK OF POSTMENOPAUSAL BREAST CANCER

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Higher levels of circulating Insulin-like Growth Factor (IGF)-I may be associated with higher risks for premenopausal breast cancer. We investigate the associations between circulating levels of IGF-I, its binding proteins (IGFBPs) -1, -2, -3, C-peptide and postmenopausal breast cancer. This is a prospective study nested in 2 Dutch cohorts. The study population included women who were postmenopausal at baseline. Breast cancer cases were identified through linkage with cancer registries. Controls were matched to cases by cohort, age, date of blood donation and place of residence. In total, 149 breast cancer cases and 333 healthy controls were included. Plasma levels of IGF-I, IGFBP-1, -2, -3 and C-peptide were measured by radioimmunoassays. Estimates of the relative risk for breast cancer associated with the quartiles of the peptides' circulating levels were obtained by conditional logistic regression. Models were adjusted for BMI, age at menarche and age at first full-term delivery. For IGF-I, the adjusted OR (95% CI) of the top *vs.* bottom quartile was 1.1 (0.6; 2.1); for IGFBP-1 it was 0.7 (0.3; 1.3); for IGFBP-2, 1.1 (0.5; 2.4); for IGFBP-3, 1.6 (0.7; 3.5), for C-peptide, 1.3 (0.7; 2.7) and for IGF-I/IGFBP-3 ratio, 1.0 (0.5; 1.8). Our data do not support an association between postmenopausal circulating levels of IGF-I, IGFBP-1, -2, -3, C-peptide and postmenopausal breast cancer.

Key words: *breast cancer; C-peptide; insulin-like growth factor-I (IGF-I); IGF binding proteins (IGFBP) -1,-2, -3; postmenopausal; prospective*

The polypeptide, hormonal family of Insulin-like Growth Factors (IGFs) consists of insulin, IGF-I and IGF-II, which have approximately 50% of their amino acids in common.[1,2] Insulin is synthesised in the beta cells of the pancreas as pro-insulin and then cleaved to form insulin and C-peptide. IGF-I is secreted mainly by the liver in response to growth hormone (GH) and is involved in the renewal regulation of epithelial cells in the breast, prostate, colon, lung and other organs.[1–6] IGF-I is inversely correlated with age.[1,7,8]

IGF-I was reported to have mitogenic and anti-apoptotic effects, and is considered to be a risk factor for carcinogenic transformation.[9,10] Substantial experimental evidence shows that GH/IGF-I axis stimulates the proliferation of normal breast epithelial cells as well as breast hyperplastic cells and breast cancer cells (*in vitro* and *in vivo*).[11–15] Moreover, it was also shown that most breast cancer cell lines and human breast cancer specimens contain IGF-I receptors in abundance.[16–18] On the other hand, some of the binding proteins that carry the IGF-I in the plasma (IGFBPs) were reported to have certain growth-regulatory effects: IGFBP-1 appears to neutralise IGF-I-dependent growth in estrogen-receptor (ER) positive breast cell lines,[19] and IGFBP-3 seems to have independent growth inhibitory and pro-apoptotic effects.[20,21]

Several, but not all, prospective population studies have provided evidence to support the presence of relationships between circulating levels of IGF-I and IGFBP-3, and the risk of prostate,[22–25] colorectal[26–30] and lung cancer.[31–33] Regarding breast cancer, prospective results differ between pre- and post-menopausal subjects. Statistically significant associations between circulating levels of IGF-I and breast cancer were previously reported in particular for younger (recruited below age 50) premenopausal women[34,35] but not for postmenopausal women. A third prospective nested case-control study from Sweden[36] did not confirm the earlier findings in young women. However, its results suggested a possible association with postmenopausal breast cancer risk, especially among users of exogenous hormones (risk estimates for the second, third and fourth quartiles of IGF-I levels as compared to the first: 3.0, 2.3 and 2.0; p for trend 0.10).[36]

The aim of our study was to investigate the associations of breast cancer with circulating levels of IGF-I, its binding proteins -1, -2, -3 and C-peptide in postmenopausal women.

MATERIAL AND METHODS

Subjects

Study subjects were derived from 2 Dutch prospective cohorts.

The Prospect-EPIC cohort. The Prospect-EPIC cohort is part of the European Prospective Investigation into Cancer and Nutrition (EPIC), co-ordinated by the International Agency for Research on Cancer (IARC) in Lyon, France.

The Prospect-EPIC cohort counts a total of 17,357 participants, all female aged 50–69 at enrolment (1993–1997). Participants were recruited through an existing national breast cancer-screening program implemented in Utrecht and vicinity (compliance rate 35%). They filled out a detailed questionnaire that contained information on dietary habits, life style and health status, and in addition they underwent a physical examination and donated a blood sample. In total, 30 ml nonfasting blood was drawn. Samples of 4 ml serum, 9 ml citrated plasma, 2 ml white blood cells (WBC) and 2 ml red blood cells (RBC) were fractionated and stored in 0.5 ml plastic straws, first directly at −80°C and several weeks later under liquid nitrogen at −196°C, for a period of 3–7 years, and on average for 5 years.[37]

The PPHV cohort. The PPHV cohort is a Dutch project aimed at monitoring risk factors for cardiovascular diseases.

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The PPHV cohort counts a total of 35,491 participants, half of them women, aged 20–59 at recruitment (1987–1991). Participants were selected randomly from the municipal registries of the cities Amsterdam, Doetinchem and Maastricht (compliance rate 57%), filled out a detailed questionnaire that contained information on dietary habits, life style and health status, underwent a physical examination and donated a blood sample. In total, 30 ml nonfasting blood samples were obtained in EDTA coated vacutainer tubes. Two 1.5 ml samplings of whole blood were stored. The remainder was centrifuged. All plasma was stored in 1.5 ml plastic tubes, buffy coat was stored in 4 ml plastic tubes and the remainder of each blood sample (mostly RBC) was stored in a similar fashion. All blood fractions were stored at −20°C for a period of 9–13 years, and on average for 11 years.[38]

Study subjects. Eligible participants (in both cohorts) were postmenopausal at enrolment (12 months or more since last menstruation), nonusers of hormone replacement therapy (HRT), nonusers of insulin and free from cancer at study enrolment. Following linkage with the local cancer registries of Utrecht, Amsterdam, Doetinchem and Maastricht as well as the National Cancer Registry, cases of first primary breast cancer were identified in both cohorts. Eligible cases were diagnosed with cancer more than 12 months following enrolment. The median interval between blood donation and diagnosis of disease was 28 months (range:14–73 months) for the prospect-EPIC cases and 72 months (range: 15–133 months) for the PPHV cases.

Cohort, age (± 1 year), place of residency (for the PPHV cohort) and date of enrolment (month and year), in order to control for the storage method and storage duration of blood samples, matched controls to each case at 2:1 ratio (+10%).

In total, we included 482 participants, 149 breast cancer cases (71 in EPIC and 78 in PPHV) and 333 controls (163 in EPIC; 170 in PPHV). All participants signed an informed consent form. The study followed the ethical standards of the Helsinki Declaration.

Laboratory assays

Plasma concentrations of IGF-I, IGFBP-1, -2, -3 and C-peptide were analysed at the Nutrition and Cancer laboratory of the International Agency for Research of Cancer, Lyon, France, by using reagents from Diagnostic Systems Laboratories (Webster, TX). IGF-I, IGFBP-1 and IGFBP-3 were measured by immunoradiometric assays, while for the measurement of IGFBP-2 and C-peptide, competitive radioimmunoassays were used. The IGF-I assay was preceded by an acid-ethanol precipitation procedure to extract IGF-I from its binding proteins.

Samples from case subjects and their matched controls were always analysed in the same batch assay kit and on the same day. To control for intra- and inter-batch variations in the measurements, 3 standard sera were inserted randomly in each batch. Intra- and inter-assay coefficients of variation (CVs) for IGF-I were 2.6% and 6.4%, respectively, at 150 ng/ml; for the binding proteins the intra- and inter-assay CVs were 3.3% and 9.9% (at 10 ng/ml) for IGFBP-1, 8.5% and 7.7% (at 400 ng/ml) for IGFBP-2 and 1.1% and 4.7% (at 4,000 ng/ml) for IGFBP-3. For C-peptide these CVs were 8.4% and 10.9% at 1.8 ng/ml.

Detection limit varied between 0.01 and 0.80 ng/ml for these assays. In 1 sample, the IGFBP-1 concentration was found to be below the detection limit, and this value was excluded from statistical analyses.

Data analysis

General characteristics of the study population are presented for both cohorts combined; as for most characteristics no substantial differences between the 2 cohorts were noted. However, mean age is also presented per cohort. Differences between cases and controls were tested by *t*-test for independent samples (for continuous variables). For categorical variables, risk estimates (and 95% confidence intervals) were computed by using unadjusted, univariate conditional logistic regression models.

Plasma levels of IGF-I are only a proxy to its target tissue levels. The type and availability of its plasma carriers (production and degradation rate of the binding proteins) determines the bioavailability of IGF-I. One of the ways to estimate the level of free (bioavailable) IGF-I is to use the (molar) ratio of IGF-I to IGFBP-3 levels, since the triple complex IGF-I/IGFBP-3/acid-labile unit is big and does not pass the capillary barrier. Mean levels of IGF-I, its binding proteins -1, -2, -3, the molar ratio of IGF-I/IGFBP-3 and C-peptide were computed separately for each cohort, as systematic differences between the cohorts were noticed. To evaluate whether variations in age or time interval since menopause could explain the observed differences, we also computed means adjusted for these variables by using linear regression models. Geometric means were computed for IGFBP-1 and C-peptide in the EPIC cohort and for IGFBP-1, IGFBP-2 and C-peptide in the PPHV cohort in order to account for their skewed distributions. The other peptides showed a normal distribution.

Within the controls, Pearson's correlation coefficients were calculated to estimate the association between continuous variables, *i.e.*, IGF-I, log-transformed IGFBP-1 and -2, IGFBP-3, IGF1/IGFBP-3 ratio, log-transformed C-peptide, age, height and BMI. Cohort-specific results did not materially differ and pooled results are presented.

We applied 2 different strategies for estimating breast cancer risk. In the first (the universal quartile approach), we determined 3 cut-off points according to the distributions of peptides' values in the total control group and thus created universal quartiles. The assumption was that both cohorts follow the same underlying distribution curve of peptides' values and that the differences noted between them might be due to random variability or methodological artefacts.

In the second approach (the cohort-specific quartile approach), we determined 3 cohort-specific cut-off points according to the distribution of controls in each cohort and created cohort-specific quartiles, which were subsequently pooled across cohorts. The hypothesis was that even if the differences noted between the cohorts resulted from substantial different distribution curves, one could still expect the relative risk of breast cancer in women at the top *vs*. the bottom quartile of each peptide to be comparable across cohorts.

Conditional logistic regression models were used to estimate the crude odds ratios (ORs) and 95% confidence intervals (95% CI) for breast cancer across quartiles. Using such models ensured that only matched sets of cases and controls from the same cohort were directly compared. Final models were adjusted for body mass index (BMI), age at menarche and age at first full term delivery on the basis of the results of univariate models.

Adding a (multiplicative) interaction term to the model covered the possibility of a modifying effect of the cohort type on the association between breast cancer and peptides' levels. The possibility of modification by age or BMI, which are both well established risk factors for breast cancer, was checked by stratifying our study population: (<60 or ≥ 60 for age and <27.0 or ≥ 27.0 for BMI).

Linear trends were estimated by the significance of *p*-values computed for the beta coefficients of bio-markers' quartiles (as a continuous variable) in the models (EGRET).

The SPSS statistical package, version 9.0[39] and the EGRET statistical package for WINDOWS[40] were used for statistical analyses. All statistical tests were 2-sided with alpha = 0.05.

RESULTS

Cases were statistically significantly younger at menarche than controls. EPIC participants were significantly older than PPHV participants ($p < 0.001$) (Table I*a*). Their mean time interval since menopause was also statistically significantly longer as compared to the PPHV participants (11.6 ± 8.5 and 6.0 ± 4.1 years, respectively, $p < 0.001$), but data was not complete for all partic-

TABLE I*a* – GENERAL CHARACTERISTICS OF THE STUDY POPULATION (2 COHORTS COMBINED)

	Cases (n = 149) mean (SD)	Controls (n = 333) mean (SD)	p Value[1]
Age at study entry (years)	57.1 (5.3)	57.1 (5.6)	0.92
EPIC[2]	59.5 (6.1)	59.4 (6.1)	0.93
PPHV[3]	55.0 (3.3)	54.8 (3.8)	0.78
Height (cm)	163.6 (6.2)	163.3 (6.0)	0.57
Weight (kg)	70.4 (11.2)	70.1 (12.2)	0.75
BMI	26.2 (3.8)	26.2 (4.2)	0.99
Age at menarche	13.3 (1.7)	13.7 (1.9)	0.03
Age at menopause (years)	48.7 (6.6)	48.4 (7.5)	0.76
Age at 1st full-term delivery (years)	27.7 (10.5)	25.7 (6.6)	0.06

[1]Independent samples *t*-test, cases *vs.* controls.—[2]EPIC participants only.—[3]PPHV participants only.

TABLE I*b* – GENERAL CHARACTERISTICS OF THE STUDY POPULATION (2 COHORTS COMBINED)

	Cases (n = 149) n (%)	Controls (n = 333) n (%)	OR (95% CI)[1]
Marital status			
Married	105 (70.5)	251 (75.4)	1.0
Widow	17 (11.4)	31 (9.3)	1.4 (0.7;2.8)
Divorced	8 (5.4)	27 (8.1)	0.7 (0.3;1.6)
Unmarried	19 (12.8)	24 (7.2)	2.2 (1.1;4.5)
Education			
Primary school completed	52 (34.9)	114 (34.4)	1.0
Technical/professional education	66 (44.3)	140 (42.3)	1.1 (0.7;1.8)
Secondary school	15 (10.1)	36 (10.9)	1.0 (0.5;1.9)
Academic education	16 (10.7)	41 (12.4)	0.8 (0.4;1.6)
Ever pregnant			
No	30 (20.1)	48 (14.4)	1.0
Yes	119 (79.9)	285 (85.6)	0.6 (0.4;1.1)
Ever breast feeding[2]			
No	12 (16.9)	28 (17.2)	1.0
Yes	48 (67.6)	116 (71.2)	1.1 (0.9;1.2)
Nonrelevant[3]	11 (15.5)	19 (11.7)	
Smoking			
Never smoker	56 (37.6)	140 (42.2)	1.0
Past smoker	45 (30.2)	93 (28.0)	1.2 (0.7;1.9)
Current smoker	48 (32.2)	99 (29.8)	1.3 (0.8;2.2)
Benign breast disease[4]			
No	21 (63.6)	55 (80.9)	1.0
Yes	12 (36.4)	13 (19.1)	1.7 (0.4;6.7)
Familial breast cancer (mother/sister)			
No	59 (83.1)	137 (84.0)	1.0
Yes	12 (16.9)	26 (15.9)	1.0 (0.5;2.0)
Ever use of OC			
No	73 (49.0)	156 (46.8)	1.0
Yes	76 (51.0)	177 (53.2)	1.1 (0.7;1.7)
Diabetes Mellitus			
No	142 (94.0)	321 (96.4)	1.0
Yes	7 (4.7)	12 (3.6)	1.8 (0.8;4.2)

[1]Odds Ratio (and 95% Confidence Intervals) computed by using unadjusted conditional logistic regression models.—[2]EPIC participants only.—[3]Nulliparous women.—[4]PPHV participants only.

ipants. Cases were significantly less-often married than controls. No statistically significant differences were noted in regard to level of education, breast feeding, smoking, prevalence of benign breast disease (PPHV), familial history of breast cancer (EPIC), use of oral contraceptives and prevalence of noninsulin-dependent diabetes mellitus between cases and controls (Table I*b*).

Mean levels of almost all peptides (except C-peptide) were systematically lower for the EPIC cohort compared to the PPHV cohort. Adjustments for age and for time interval since menopause explained the differences only partially and did not materially change the results (data not shown). Since the distribution of some of the peptides was skewed, we present their medians rather than their mean levels. Median serum concentrations in ng/ml for the EPIC and PPHV cohorts, respectively, were 146.6 and 174.6 for IGF-I, 11.6 and 20.9 for IGFBP-1, 396.0 and 577.6 for IGFBP-2, 3,151.3 and 3,799.0 for IGFBP-3, 0.17 and 0.17 for IGF-I/IGFBP-3 and 2.7 and 2.6 for C-peptide.

Within the control group, IGF-I was positively correlated with IGFBP-3 (0.6, p=0.01). The molar ratio IGF-I/IGFBP-3 was significantly correlated both with IGF-I (0.9, p=0.01) and IGFBP-3 levels (0.2, p=0.01) (Table II).

As indicated in the Methods section, 2 strategies were applied to assess the risk estimates for breast cancer: the universal quartile approach and the cohort-specific quartile approach. As results were very similar for both, only the results of the first strategy are presented here (Table III). the results were not statistically significant for any of the peptides. Risk estimates were adjusted for BMI, age at menarche and age at first full term delivery. Odds ratio for breast cancer risk between the top *vs*. bottom quartiles of IGF-I were 1.1 (95% CI 0.6; 2.1). Results for IGFBP-3 were 1.6 (0.7; 3.5) and for C-peptide were 1.3 (0.7; 2.7). Further adjustments for age at menopause, time interval since menopause, diabetes, height, weight, IGF-I levels (for IGFBP-3), IGFBP-3 levels (for IGF-I), IGF-I and IGFBP-3 levels (for C-peptide), and C-peptide levels

TABLE II – PEARSON'S CORRELATION COEFFICIENTS MATRIX (n = 333, TOTAL CONTROLS)

	ln IGFBP-1	ln IGFBP-2	IGFBP-3	ln C-peptide	IGF-I/BP-3[1]	Age	Height	BMI
IGF I	−0.06	−0.02	0.58**	−0.02	0.88**	−0.30**	0.03	−0.09
ln IGFBP-1	—	0.37**	−0.09	−0.40*	−0.00	−0.17**	−0.09	−0.34**
ln IGFBP-2	—	—	−0.03	−0.31**	−0.02	−0.19**	−0.03	−0.31**
IGFBP-3	—	—	—	0.07	0.15**	−0.22**	0.05	0.03
ln C-peptide	—	—	—	—	−0.08	0.22**	0.08	0.29**
IGF-I/BP-3	—	—	—	—	—	−0.22**	−0.01	−0.14*
Age	—	—	—	—		—	−0.09	0.18**
Height	—	—	—	—		—	—	−0.08

*Correlation is significant at the 0.05 level (two-tailed).—**Correlation is significant at the 0.01 level (two-tailed).—[1]refers to the molar ratio of IGF-1/IGFBP-3.

TABLE III – ORS (95% CI) FOR BREAST CANCER BY QUARTILES OF IGF-I, IGFBP-1 -2 -3, IGF-1/IGFBP-3 AND C-PEPTIDE[1]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
IGF-I	*41;83**	*34;83**	*32;85**	*42;80**
Median exposure ng/ml	100.9	141.7	176.6	242.0
	1.0[2]	0.8 (0.4;1.4)	0.7 (0.4;1.2)	1.0 (0.6;1.8)
	1.0[3]	0.8 (0.4;1.6)	0.7 (0.3;1.5)	1.1 (0.6;2.1)
	1.0[4]	0.5 (0.3;1.1)	0.5 (0.2;1.0)	0.7 (0.3;1.5)
IGFBP-1	*44;84**	*42;82**	*31;83**	*31;84**
Median exposure ng/ml	5.2	11.6	21.5	45.2
	1.0[2]	1.1 (0.7;1.9)	0.7 (0.5;1.6)	0.7 (0.4;1.2)
	1.0[3]	1.2 (0.6;2.2)	1.1 (0.6;2.0)	0.7 (0.3;1.3)
IGFBP-2	*33;83**	*32;83**	*45;83**	*39;83**
Median exposure ng/ml	260.7	407.5	567.2	797.7
	1.0[2]	1.0 (0.5;1.8)	1.4 (0.7;2.5)	1.2 (0.7;2.3)
	1.0[3]	0.7 (0.3;1.4)	1.0 (0.5;2.1)	1.1 (0.5;2.4)
IGFBP-3	*35;82**	*42;83**	*26;83**	*46;83**
Median exposure ng/ml	2,829.5	3,297.2	3,752.2	4,275.8
	1.0[2]	1.3 (0.7;2.3)	0.7 (0.4;1.3)	1.4 (0.7;2.6)
	1.0[3]	1.5 (0.8;2.8)	0.6 (0.3;1.3)	1.6 (0.7;3.5)
	1.0[5]	0.9 (0.4;1.8)	0.6 (0.2;1.2)	1.4 (0.6;3.4)
IGF-I/IGFBP-3	*43;84**	*31;82**	*38;83**	*37;82**
Median exposure ng/ml	0.12	0.15	0.18	0.23
	1.0[2]	0.7 (0.4;1.3)	0.9 (0.5;1.5)	0.9 (0.5;1.5)
	1.0[3]	0.7 (0.4;1.3)	0.9 (0.5;1.7)	1.0 (0.5;1.8)
C peptide	*31;83**	*35;83**	*44;83**	*39;84**
Median exposure ng/ml	1.3	2.2	3.4	6.0
	1.0[2]	1.0 (0.6;1.9)	1.3 (0.7;2.3)	1.2 (0.7;2.1)
	1.0[3]	1.3 (0.6;2.6)	1.2 (0.6;2.4)	1.3 (0.7;2.7)

[1]Quartiles are determined by universal cut-off points based on total controls' distribution.—*Numbers of cases; controls (for one case IGFBP-1 levels were below detection limits; controls do not always sum up to the total of 333 due to missing data in some of the adjustment variables.—[2]Crude (matched for cohort, age at enrollment, date of blood sampling and place of residence).—[3]Adjusted for BMI, age at menarche, age at first full term delivery.—[4]Adjusted for BMI, age at menarche, age at first full term delivery and IGFBP-3 levels.—[5]Adjusted for BMI, age at menarche, age at first full term delivery and IGF-I levels.

(for IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3) did not materially change the results and are not presented here.

Cohort type, age or BMI did not modify the association between breast cancer and peptides' plasma levels.

DISCUSSION

The results of the present prospective cohort study do not show associations between circulating levels of IGF-I, IGFBP-1, -2, -3, C-peptide and subsequent breast cancer in postmenopausal middle-aged and elderly Dutch women.

The main advantages of the present study are its design, the statistical power and its completeness. The prospective nature of the study ensures that blood samples were always donated long before the occurrence of the disease. Furthermore, all our cases were diagnosed 14 or more months following enrolment. The combining of 2 cohorts expanded the number of breast cancer cases and enhanced the statistical power so that the chances of detecting a statistically significant risk increase of 150% between the highest *vs*. the lowest quartiles of IGF-I were around 90%. Similarly, we could detect a breast cancer risk reduction of 50% between the top *vs*. bottom quartiles of IGFBP-3 or a doubling of breast cancer risk between the top *vs*. bottom quartiles of C-peptide at a power level of 85–90%. In addition, the completeness of the Regional and National Cancer Registries reduced the possibility of misclassifications.

The differences noted between the mean levels of peptides in the prospect-EPIC and the PPHV cohorts (generally lower in EPIC except for C-peptide) could have reflected 2 genuinely different populations, random variability or an artefact. As both cohorts are population based and consist of mostly Caucasian, Dutch women with nonselective and heterogeneous demographic and anthropometric backgrounds, a genuine difference in peptides' mean levels is not very likely. Random variability does not seem to account for the systematic and statistically significant differences either. Consequently, an artefact seems plausible. Blood handling and storing were different for both cohorts; the use of citrate in the EPIC plasma samples may have induced dilution effect.[41] Use of EDTA, as in the PPHV plasma samples, does not have such an effect.[42] Additionally, EPIC plasma samples were stored in heat-sealed plastic straws, while PPHV samples were stored in plastic tubes covered with manually screwed-on plastic cups. During the storage period, which was longer for the PPHV samples, micro-evaporation of water droplets might have resulted in a further increase of

peptide levels in the PPHV samples. A laboratory error is not very likely since blood samples of cases and their matched controls were always analysed in the same batch, and samples from both cohorts were mixed in each batch. However, the use of conditional logistic regression models, which ensures that each case is directly compared to its matching cohort-specific controls, accounts for the observed differences.

Although we have not studied the effects of storage duration and temperature on the peptides' stability, peptide levels in both our cohorts are similar to those reported previously for other prospective cohorts. Median plasma level of IGF-I (in ng/ml) in postmenopausal controls was 153 in the Nurses' Health Study[34] and 156 (Umea) and 176 (Malmo) in the Swedish study,[36] while in our study IGF-I medians were 147 in EPIC and 175 in PPHV. Likewise, median levels of IGFBP-3 (in ng/ml) for controls in the Swedish study[36] were 3,604 (Umea) and 3,643 (Malmo), while in our study they were 3,151 for EPIC and 3,799 for PPHV. For C-peptide, mean level for postmenopausal controls in the New York's University Women's Health Study[35] was 4.0 ± 2.0 ng/ml, while corresponding values in our study were 3.7 ± 0.6 and 3.2 ± 2.0 ng/ml for EPIC and PPHV, respectively.

The results of our study with regard to IGF-I correspond with previous studies.[34–36]

Our findings in regard with C-peptide are in accordance with previous prospective studies. Three previous case-control studies reported a positive, significant association between levels of C-peptide or insulin, and breast cancer risk, in premenopausal as well as postmenopausal[43,44] subjects. However, their results could have been biased by their retrospective design. Two prospective studies could not detect a relationship between nonfasting C-peptide plasma levels and postmenopausal breast cancer,[35] or insulin plasma levels and breast cancer risk.[36] Obtaining accurate insulin levels usually requires long fasting. It should be noted that only 55.5% of the participants in the study by Kaaks et al.[36] donated their blood samples after fasting for 8+ hr.[36] We obtained data regarding time since last meal and drink for the Prospect-EPIC participants only and therefore could adjust only in this cohort for this variable. Doing so, however, did not materially alter the results (data not shown), although p-values for trend were borderline significant ($p=0.06$) for C-Peptide. Null effects were noted when we focused on over-weight and obese women (BMI = or >27) or on the subgroup with higher (above median) levels of C-peptide.

The question is why risks might be different according to menopausal status.

IGF-I may enhance cancer risk independently through promoting cell proliferation and inhibiting apoptosis.[6,46,47] It could also act synergistically with other hormones (*i.e.*, insulin) and/or other known risk factors.[9,10] Differences in hormonal and metabolic environments between pre- and post-menopausal women might explain the variable risk associations reported. IGF-I circulating levels are inversely correlated with age (1,8); endogenous estradiol enhances the stimulatory action of IGF-I in breast cells.[46] Hence, postmenopausal women, with lower circulating levels of IGF-I, and lower endogenous estradiol levels, which decrease the sensitivity of their breast cells to the stimulatory effects of IGF-I, might be less susceptible to its effects than premenopausal women.

Insulin, which was previously suggested as a risk factor for breast cancer,[47,48] increases the bioavailability of IGF-I by enhancing its synthesis and by inhibiting the production of IGFBP-1 and -2 in the liver and other tissues.[47,49] Furthermore, insulin and IGF-I both stimulate the synthesis of sex steroids and inhibit the synthesis of sex-steroids binding proteins, thus exposing women with higher insulin and IGF-I levels to higher levels of sex steroids as well, a known risk factor for breast cancer.[47] Higher insulin levels in women, both pre- and post-menopausal, may possibly enhance their risk for breast cancer through the independent and synergistic actions of IGF-I and insulin. However, evidence from human studies, including the present one, is inconclusive.

In conclusion, the results of our study do not support the presence of a relationship between postmenopausal circulating levels of IGF-I and its binding proteins, or postmenopausal C-peptide levels, and risk of breast cancer.

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