RESEARCH COMMUNICATION

Insulin, Insulin-like Growth Factor-I and Breast Cancer Risk in Japanese Women

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Abstract

To evaluate the effects of glucose metabolism related factors, such as insulin and insulin-like growth-factors (IGFs), on breast cancer development among Japanese women, we conducted a case-referent study comparing 187 women presenting with operable breast cancer and 190 women of the same age having no breast cancer. Odds ratios (OR) and 95% confidence intervals (95%CI) were determined by multiple logistic regression analysis.

In the present study, no association in risk was observed with increasing levels of IGF-I or IGF binding protein-3 (IGFBP-3), before or after adjustment these factors. However, a suggestion of a positive association of an increased breast cancer risk was evident in postmenopausal women with elevated plasma insulin levels, particularly those with BMI>23.07. The OR for plasma insulin in the top tertile was 4.48 (95%CI:1.07-18.7) compared to the bottom tertile. For C-peptide, there was a similar positive association, with a corresponding OR of 2.28. In addition, we observed strong links between plasma insulin, C-peptide levels and estrogen receptor (ER) negative breast cancer, with ORs of 2.79(95%CI:1.09-7.16), and 2.52 (95%CI:0.91-6.97) respectively, for the top versus bottom tertiles. In conclusion, the present study suggested that plasma insulin level is a predictor of postmenopausal breast cancer in obese women and ER negative breast cancer. Additional studies are needed to clarify the role of glucose metabolism pathways in breast cancer development and interaction of IGF systems.

Key Words: insulin - IGF-I - breast cancer - BMI - hormone receptor status

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Introduction

The insulin-like growth factor (IGF) system is a key growth regulatory pathway in breast cancer. Thus various components have been directly or indirectly implicated in neoplasia of the mammary glands and it is essential to gain a better understanding of their role in its etiology. IGF-I, a mitogenic and antiapoptotic peptide, can affect the proliferation of breast epithelial cells, but little is known about its relationship for breast cancer in Asian women since most previous epidemiological studies have been conducted in the Caucasian. Energy intake, body mass index (BMI) and physical activity all appear to affect blood levels of IGFs and IGF-binding proteins (IGFBPs). As animal studies indicate that IGF-I levels in the circulation are positively correlated with total energy intake (Dunn et al., 1997), it is possible that the differences in dietary habits between Japanese and Caucasian population exert on influence.

IGF-I is a peptide hormone with a high degree of homology with proinsulin. Together with IGF-I, insulin is central to the regulation of anabolic processes as a function of available energy with a key role in modulating IGF-I bioactivity. Estradiol is generally regarded as the most important hormonal promoter of breast cancer development, but insulin may also have significance, partly by increasing the availability of IGF-I within the ovaries (Poretsky and Kalin, 1987). The effects of insulin on steroid hormone metabolism even may be mediated entirely by increased physiologic activity of IGF-I (Poretsky and Kalin, 1987). Insulin also may play an etiologic role in association with

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obesity, which increases insulin resistance and, moreover, decreases serum levels of sex hormone binding globulin (SHBG) and thus elevates estrogen availability (Kaya et al., 1991). We have provided evidence that gain in body mass as an adult is a predictor of breast cancer risk among postmenopausal Japanese women (Hirose et al., 1999). Although the risk impact related to insulin resistance and/or type 2 diabetes, such as obesity, has addressed (Kahn and Flier, 2000; Mayer-Davis et al., 1997; Storlien et al., 1996), few epidemiological studies have directly investigated the relationship between related hormone levels and breast cancer risk. We have report the results of plasma insulin and C-peptide (a marker of pancreatic insulin secretion) measurements in a case-control study to evaluate the relationship between such factors and breast cancer risk.

Materials and Methods

Study subjects

As we have reported in detail (Hirose et al., 1999; Tajima et al., 2000), the hospital-based epidemiologic research program at Aichi Cancer Center (HERPACC) study, featuring a self-administered questionnaire completed by first-visit outpatients at the Aichi Cancer Center Hospital (ACCH), has been ongoing for some time. Before diagnosis subjects complete a questionnaire with items concerning family history of cancer, age at menarche and menopause, reproductive history, height and weight. Among women referred to the Department of Breast Surgery in ACCH between November, 2000 and September, 2002, 187 women presenting with operable breast cancer aged 30 to 78 years, together with 190 women with no breast cancer and belonging to the same age group, were studied. After providing informed consent, study subjects donated a 7 ml sample of peripheral venous blood on the first-visit day. Plasma was separated soon after collection, and then stored at -80 ^{o}C until laboratory analyses could be conducted.

Specimen Measurement

Trained staff, blind to the case-reference status, assayed all samples at a single laboratory (SRL,Hachioji). Plasma concentrations of IGF-I and IGFBP-3 were measured by immunoradiometric assay, using commercially available kits (Dai-ichi Radioisotope Lab., Tokyo). Total IGF-I can be measured with kits by separating IGFs from their binding proteins. The manufacturer validated the IGF assays against methods using acid-ethanol extraction and/or acid-column chromatography. Plasma insulin and C-peptide were measured enzymatically using a two-step sandwich-type EIA assay kit from Eiken Chemical Co., Ltd.

Both estrogen receptor (ER) and progesterone receptor (PR) levels in breast tissue were determined using commercially available enzyme immunoassay kits. In this study, the receptor status was known for 88.9% of cases, with ER and PR positive rates of 68.1% and 59.4%, respectively.

Table 1. Comparison of Characteristics between Breast Cancer Patients and Referents

Variable	Cases (n=187) Mean (SD)	Referents (n=190) Mean (SD)	P (a)
Age (year)	52.7 (10.6)	52.2 (10.9)	0.679
Age at menarche (year)	13.4 (1.5)	13.4 (2.0)	0.912
Age at first full-term pregnancy	26.5 (3.7)	25.7 (3.4)	0.052
BMI (kg/m^2)			
Premenopause	21.5 (3.0)	21.3 (2.3)	0.596
Postmenopause	23.1 (2.9)	23.1 (3.4)	0.936
	Percent	Percent	P (b)
Menopausal status			
Pre	47.1	41.6	
Post	52.9	58.4	0.284
Number of parity			
1	20.1	15.6	
2	54.4	58.1	
3+	25.4	26.3	0.564
Family history			
No	90.7	94.0	
Yes	9.3	6.0	0.226
History of diabetes (self-reported)			
No	95.5	97.9	0.214
Yes	4.5	2.2	

a: t-test, b:χ2 test

Statistical Analysis

Logistic regression was used to obtain odds ratios (OR) and 95% confidence intervals (95% CI) as estimates of relative risk. Tertiles were defined according to the distribution among reference subjects. To control for potential confounding by established risk factors for breast cancer, we carried out multivariate analyses using a model including age, family history, age at menarche, parity, age at first delivery and BMI (weight / height^2 (kg/m^2)). In the multivariate analysis, IGFBP-3 and IGF-I were mutually adjusted as these molecules might positive correlated with regard to biological function. Calculations were performed using the SAS (SAS Institute) LOGISTIC procedure. The statistical significance of the differences between ER (or PR)-positive vs. ER (or PR)-negative was determined by case-to-case comparisons, using the multiple logistic regression model.

Results

Table 1 summarizes data for characteristics compared between breast cancer patients and referents. There were no statistically significant differences with respect to age, age at menarche, BMI, parity, family history of cancer and medical history of diabetes. Compared with referents, more cases had a later age at first full-term pregnancy (P<0.052).

Ranges and median concentrations of IGF-I, IGFBP-3, insulin and C-peptide in plasma are given in Table 2. No significant differences were found with the menopausal status, although postmenopausal cases had slightly higher levels of insulin than referents. IGF-I levels in plasma were inversely correlated with age, with similar trends in cases (Spearman's r=-0.34; P<0.0001) and references (r=-0.36;P<0.001). Insulin was positively correlated with BMI, this being particularly remarkable in the case group (r=0.39;P<0.0001). IGF-I was strongly correlated with IGFBP-3 in both cases (r=0.53; P<0.0001) and referents (r=0.48;P<0.0001).

Table 3-a summarizes results from conditional logistic regression analyses for all women. For plasma measurements among all women, none of the associations were significant. Associations were also examined in subgroups of women stratified by menopausal status (Tables 3-b and 3-c). With or without adjustment, high insulin levels were strongly associated with increased risk among postmenopausal women but not premenopausal women. The adjusted OR for the top versus bottom tertiles was 2.43(95%CI:1.06-5.58) among postmenopausal women (Table 3-c). Although the ORs for postmenopausal breast cancer were somewhat elevated with C-peptide, none of the associations or trends was statistically significant.

To assess the joint effects of insulin or C-peptide concentrations with BMI, we compared the risk of breast cancer with these variables in subgroups of postmenopausal women stratified by BMI (Table 4). There was a positive association between insulin concentration and breast cancer risk in heavier postmenopausal women. Plasma insulin in the top tertile was associated with an OR of 4.48 (95%CI:1.07-18.7) compared to the bottom tertile. For C-peptide, there was a similar positive association, with an OR of 2.28, for the top versus the bottom tertile.

Tables 5-a and 5-b summarize ORs for breast cancer among all women according to hormone receptor status. The association between plasma insulin concentration and ER-

Table 2 Plasma Levels of IGF-I, IGFBP-3, Insulin and C-peptide for Breast Cancer Cases and Referents

Variable	Cases		Referents		P a
	No.	Median(range)	No.	Median(range)	
All women					
IGF-I (ng/ml)	187	170 (73-390)	190	180 (59-420)	0.79
IGFBP-3 (μ g/ml)	187	2.87 (1.68-3.94)	190	2.89 (1.63-3.90)	0.79
insulin (μ U/ml)	187	7.64 (1.54-122.0)	190	7.26 (1.53-87.7)	0.79
C-peptide (ng/ml) b	187	0.50 (0.08-3.46)	189	0.50 (0.11-3.68)	0.86
Premenopausal women					
IGF-I (ng/ml)	88	190 (86-390)	79	190 (95-420)	0.23
IGFBP-3 (μ g/ml)	88	2.86 (2.36-3.41)	79	2.89 (2.27-3.41)	0.23
insulin (μ U/ml)	88	6.23 (1.53-86.0)	79	6.12 (1.53-73.9)	0.23
C-peptide (ng/ml)	88	0.39 (0.08-2.72)	78	0.39 (0.11-2.62)	0.19
Postmenopausal women					
IGF-I (ng/ml)	99	160 (73-310)	111	160 (59-370)	0.16
IGFBP-3 (μ g/ml)	99	2.89 (1.68-3.94)	111	2.89 (1.63-3.90)	0.16
insulin (μ U/ml)	99	9.75 (2.50-122.0)	111	7.42 (2.57-87.4)	0.16
C-peptide (ng/ml)	99	0.63 (0.20-3.46)	111	0.59 (0.21-3.68)	0.16

a Wilcoxon signed rank test.

b One sample was not measured due to lack of plasma.

Table 3-a. ORs for Breast Cancer by Tertiles of Plasma Measurements for all Women

Variable		Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)
IGF-I	=<150	72/69	1.00 (reference)	1.00 (reference)
(ng/ml)	150-200	45/55	0.80 (0.47-1.34)	0.74 (0.40-1.37)
	>=200	70/66	1.06 (0.64-1.74)	1.00 (0.53-1.89)
	p for trend		0.837	0.967
IGFBP-3	=<2.74	66 / 65	1.00 (reference)	1.00 (reference)
(μ g/ml)	2.74 - 3.02	71 / 62	1.15 (0.70-1.87)	1.15 (0.65-2.05)
	>= 3.02	50 / 63	0.79 (0.47-1.30)	0.76 (0.40-1.45)
	p for trend		0.376	0.403
Insulin	=<5.06	52 / 63	1.00 (reference)	1.00 (reference)
(μ U/ml)	5.06 - 10.2	65 / 62	1.26 (0.76-2.10)	1.24 (0.70-2.20)
	>= 10.2	70 / 65	1.29 (0.78-2.14)	1.21 (0.68-2.16)
	p for trend		0.332	0.532
C-peptide	=<0.36	54 / 63	1.00 (reference)	1.00 (reference)
(ng/ml)	0.36 - 0.68	70 / 63	1.28 (0.77-2.13)	1.30 (0.73-2.31)
	>=0.68	63 / 64	1.13 (0.67-1.91)	0.97 (0.53-1.77)
	p for trend		0.676	0.864

OR1:adjusted for age
OR2:adjusted for age, family history, age at menarche, parity, age at first delivery, BMI, and either IGFBP-3 or IGF-I (where applicable).

negative breast cancer risk increased with an OR of 2.79 (95%CI:1.09-7.16) between the top and bottom tertiles (Table 5-a). The corresponding OR with plasma C-peptide was similar, although the association was not statistically significant (OR=2.52, 95%CI:0.91-6.97). In contrast, no significant variation in the association of breast cancer risk with plasma measurements was apparent for the PR status (Table 5-b).

Table 3-b. ORs for Breast Cancer by Tertiles of Plasma Measurements for Premenopausal Women

Variable		Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)
IGF-I	=<160	33 / 24	1.00 (reference)	1.00 (reference)
(ng/ml)	160-210	23 / 25	0.70 (0.32-1.52)	0.82 (0.30-2.20)
	>=210	32 / 30	0.88 (0.41-1.88)	0.86 (0.32-2.29)
	p for trend		0.741	0.783
IGFBP-3	=<2.74	30 / 25	1.00 (reference)	1.00 (reference)
(μ g/ml)	2.74 - 2.99	38 / 27	1.25 (0.60-2.62)	1.34 (0.53-3.40)
	>= 2.99	20 / 27	0.68 (0.30-1.54)	0.88 (0.31-2.49)
	p for trend		0.385	0.762
Insulin	=<4.67	32 / 26	1.00 (reference)	1.00 (reference)
(μ U/ml)	4.67 - 9.84	29 / 25	1.02 (0.48-2.17)	0.85 (0.34-2.13)
	>= 9.84	27 / 28	0.76 (0.36-1.61)	0.55 (0.22-1.36)
	p for trend		0.482	0.195
C-peptide	=<0.30	25 / 26	1.00 (reference)	1.00 (reference)
(ng/ml)	0.30 - 0.55	38 / 26	1.53 (0.72-3.21)	1.27 (0.49-3.28)
	>=0.55	25 / 27	0.97 (0.44-2.10)	0.85 (0.32-2.23)
	p for trend		0.925	0.662

OR1: adjusted for age
OR2: adjusted for age, family history, age at menarche, parity, age at first delivery, BMI, and either IGFBP-3 or IGF-I (where applicable).

Table 3-c. ORs for Breast Cancer by Tertiles of Plasma Measurements for Postmenopausal Women

Variable		Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)
IGF-I	=<130	29 / 36	1.00 (reference)	1.00 (reference)
(ng/ml)	130-170	35 / 40	1.15 (0.58-2.26)	0.90 (0.39-2.05)
	>=170	35 / 35	1.48 (0.73-3.02)	1.30 (0.48-3.42)
	p for trend		0.277	0.594
IGFBP-3	=<2.68	35 / 35	1.00 (reference)	1.00 (reference)
(μ g/ml)	2.68 - 3.03	31 / 38	0.84 (0.43-1.65)	0.71 (0.32-1.57)
	>= 3.03	33 / 38	0.91 (0.47-1.77)	0.60 (0.24-1.47)
	p for trend		0.784	0.266
Insulin	=<5.19	16 / 37	1.00 (reference)	1.00 (reference)
(μ U/ml)	5.19 - 11.0	43 / 36	2.69 (1.29-5.64)	2.56 (1.14-5.78)
	>= 11.0	40 / 38	2.41 (1.15-5.05)	2.43 (1.06-5.58)
	p for trend		0.033	0.054
C-peptide	=<0.43	21 / 37	1.00 (reference)	1.00 (reference)
(ng/ml)	0.43 - 0.72	37 / 36	1.75 (0.86-3.55)	2.11 (0.94-4.72)
	>=0.72	41 / 38	1.83 (0.91-3.68)	2.00 (0.89-4.52)
	p for trend		0.105	0.125

OR1: adjusted for age
OR2: adjusted for age, family history, age at menarche, parity, age at first delivery, BMI, and either IGFBP-3 or IGF-I (where applicable).

Discussion

The present case-referent study conducted to examine the association of breast cancer with insulin, c-peptide, IGF-I and IGFBP-3 levels in plasma among Japanese women, demonstrated a positive association of plasma insulin level with breast cancer risk in heavier (BMI>23.07) postmenopausal women, especially in cases with an ER negative status.

Fasting blood samples are needed for measuring blood insulin or C-peptide, because insulin secretion is heavily influenced by recent meals. The non-fasting state of our study subjects was chosen mainly for practical reasons, but provided insight into normal daily conditions and most blood samples were drawn at least 2 hours after eating. A major strength of the present study is that all blood samples were collected before diagnosis. Thus, the observed case-reference difference in level of plasma insulin and C-peptide cannot be attributed to any influence of resultant treatment.

Our finding that high insulin levels are associated with an increased risk of breast cancer among heavier postmenopausal women needs to be interpreted cautiously because of the low sample size and limitations of blood collection. It is possible that the effect of BMI in the present study is explained by both the relation between increased body fat and insulin resistance and secretion of IGF-I (De

Table 4. Joint Effects of Insulin or C-peptide Concentration and BMI on the Risk of Breast Cancer among Postmenopausal Women

Variable		BMI =<23.07 (median)			BMI >23.07 (median)		
		Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)	Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)
Insulin	=<5.19	13/24	1.00 (reference)	1.00 (reference)	3/13	1.00 (reference)	1.00 (reference)
(μ U/ml)	5.19 - 11.0	20/16	2.30 (0.89-5.96)	1.43 (0.49-4.18)	23/20	4.88 (1.21-19.7)	6.13 (1.40-26.8)
	>= 11.0	17/16	2.09 (0.79-5.55)	1.45 (0.48-4.44)	23/22	4.42 (1.10-17.7)	4.48 (1.07-18.7)
	p for trend		0.129	0.506		0.092	0.127
C-peptide	=<0.43	16/25	1.00 (reference)	1.00 (reference)	5/12	1.00 (reference)	1.00 (reference)
(ng/ml)	0.43 - 0.72	15/19	1.19 (0.47-3.02)	0.93 (0.32-2.70)	22/17	3.08 (0.91-10.5)	4.59 (1.12-18.8)
	>=0.72	19/12	2.43 (0.93-6.37)	1.86 (0.61-5.69)	22/26	2.01 (0.61-6.61)	2.28 (0.59-8.74)
	p for trend		0.077	0.305		0.532	0.61

OR1:adjusted for age
OR2:adjusted for age, family history, age at menarche, parity and age at first delivery

Table 5-a ORs for Breast Cancer among all Women According to Estrogen Receptor Status

Variable		ER (-) Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)	ER (+) Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)	P value for inter-case comparison a
IGF-I	=<150	15/69	1.00 (reference)	1.00 (reference)	49/69	1.00 (reference)	1.00 (reference)	
(ng/ml)	150-200	13/55	1.09 (0.47-2.51)	1.07 (0.38-2.97)	29/55	0.77 (0.43-1.40)	0.76 (0.37-1.54)	
	>=200	25/66	1.76 (0.81-3.81)	1.99 (0.73-5.47)	35/66	0.80 (0.45-1.45)	0.70 (0.33-1.47)	
	p for trend		0.141	0.153		0.438	0.341	0.413
IGFBP-3	=<2.74	17/65	1.00 (reference)	1.00 (reference)	42/65	1.00 (reference)	1.00 (reference)	
(μ g/ml)	2.74 - 3.02	20/62	1.21(0.58-2.53)	0.82 (0.33-2.03)	43/62	1.10 (0.63-1.93)	1.19 (0.61-2.31)	
	>= 3.02	16/63	0.96(0.44-2.06)	0.70 (0.27-1.83)	28/63	0.76 (0.42-1.39)	0.81 (0.38-1.73)	
	p for trend		0.911	0.466		0.405	0.602	0.334
Insulin	=<5.06	12 / 63	1.00 (reference)	1.00 (reference)	36 / 63	1.00 (reference)	1.00 (reference)	
(μ U/ml)	5.06 - 10.2	14 / 62	1.20 (0.51-2.80)	1.97 (0.71-5.44)	41 / 62	1.20 (0.67-2.14)	0.92 (0.48-1.76)	
	>= 10.2	27 / 65	2.25 (1.04-4.86)	2.79 (1.09-7.16)	36 / 65	0.96 (0.53-1.75)	0.78 (0.39-1.53)	
	p for trend		0.031	0.033		0.900	0.468	0.078
C-peptide	=<0.36	11 / 63	1.00 (reference)	1.00 (reference)	39 / 63	1.00 (reference)	1.00 (reference)	
(ng/ml)	0.36 - 0.68	19 / 63	1.87 (0.81-4.32)	2.72 (1.00-7.38)	39 / 63	0.98 (0.55-1.75)	0.80 (0.41-1.55)	
	>=0.68	23 / 64	2.29 (0.99-5.25)	2.52 (0.91-6.97)	35 / 64	0.89 (0.48-1.63)	0.68 (0.34-1.38)	
	p for trend		0.056	0.108		0.698	0.286	0.116

OR1: adjusted for age and menopausal status
OR2: adjusted for age, menopausal status, family history, age at menarche, parity, age at first delivery and BMI
a: Likelihood ratio test for differences between ER-positve and ER-negative breast cancer cases, adjusted for all other covariates.

Table 5-b ORs for Breast Cancer among all Women According to Progesterone Receptor Status

Variable		PR (-) Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)	PR (+) Number Case/Reference	OR 1 (95%CI)	OR 2 (95%CI)	P value for inter-case comparison a
IGF-I	=<150	23 / 69	1.00 (reference)	1.00 (reference)	40 / 69	1.00 (reference)	1.00 (reference)	
(ng/ml)	150-200	15 / 55	0.87 (0.41-1.84)	0.92 (0.37-2.30)	27 / 55	0.90 (0.48-1.67)	0.97 (0.46-2.06)	
	>=200	29 / 66	1.48 (0.74-2.95)	1.57 (0.61-4.03)	31 / 66	0.81 (0.43-1.51)	0.70 (0.32-1.56)	
	p for trend		0.256	0.325		0.501	0.383	0.213
IGFBP-3	=<2.74	22 / 65	1.00 (reference)	1.00 (reference)	37 / 65	1.00 (reference)	1.00 (reference)	
(μ g/ml)	2.74 - 3.02	21 / 62	1.02 (0.51-2.06)	0.61 (0.25-1.49)	42 / 62	1.20 (0.67-2.14)	1.28 (0.64-2.55)	
	>= 3.02	24 / 63	1.11 (0.56-2.18)	0.87 (0.35-2.17)	19 / 63	0.61 (0.31-1.18)	0.58 (0.26-1.32)	
	p for trend		0.769	0.835		0.188	0.224	0.558
Insulin	=<5.06	18 / 63	1.00 (reference)	1.00 (reference)	30 / 63	1.00 (reference)	1.00 (reference)	
(μ U/ml)	5.06 - 10.2	20 / 62	1.10 (0.53-2.28)	1.39 (0.57-3.39)	35 / 62	1.33 (0.71-2.47)	1.07 (0.54-2.13)	
	>= 10.2	29 / 65	1.51 (0.76-3.00)	1.88 (0.82-4.31)	33 / 65	1.15 (0.61-2.16)	0.83 (0.41-1.71)	
	p for trend		0.230	0.136		0.677	0.614	0.263
C-peptide	=<0.36	17 / 63	1.00 (reference)	1.00 (reference)	33 / 63	1.00 (reference)	1.00 (reference)	
(ng/ml)	0.36 - 0.68	24 / 63	1.36 (0.66-2.80)	1.57 (0.67-3.67)	34 / 63	1.06 (0.57-1.98)	0.91 (0.45-1.83)	
	>=0.68	26 / 64	1.42 (0.69-2.93)	1.49 (0.63-3.52)	31 / 64	1.06 (0.55-2.02)	0.72 (0.34-1.53)	
	p for trend		0.362	0.400		0.869	0.394	0.376

OR1: adjusted for age and menopausal status
OR2: adjusted for age, menopausal status, family history, age at menarche, parity, age at first delivery and BMI
a: Likelihood ratio test for differences between PR-positve and PR-negative breast cancer cases, adjusted for all other covariates.

Perola et al., 1993). Insulin has a mitogenic effect on mammary epithelium cells (Milazzo et al., 1992; Cullen et al., 1990; Osborne et al., 1990) and insulin and IGF-I can synergistically stimulate mammary cell proliferation. It has also been observed that insulin receptor may be overexpressed in human breast cancer (Shafie et al., 1977; Pekonen et al., 1988; Papa et al., 1997; Webster et al., 1996). By decreasing the levels of SHBG, insulin also can lead to increased availability of free estradiol (Henderson and Feigelson, 2000). Furthermore, insulin can stimulate the

synthesis of both androgen and estrogen in ovarian tissue (Stoll, 1998). Therefore, the link between insulin and breast cancer risk may be through the role of this hormone in regulation of the levels, bioavailability, and effects of both IGF-I and estrogen.

The presence of both ER and PR in breast cancer tissue has been recognized as an important prognostic factor for the clinical course. However, it is not yet clear whether mammary neoplasms with differing hormone receptor status represent etiologically distinct forms of the disease with different risk factor profiles. In our previous study with HERPACC data, some evidence was obtained that certain risk factors, including reproductive factors, may differ by PR status, but not by ER status (Yoo et al., 1997). A high level of insulin was, however, associated with increased odds for ER-negative breast cancer, although not statistically significant ($P<0.078$). The present study indicated the association between breast cancer risk and high insulin to not differ significantly with the PR status. Although we did not find a statistically significant association between serum C-peptide levels, regarded as a better estimate of insulin secretion than levels of insulin itself, and cancer, some indication was evident for ER negative breast cancer cases. In hormone responsive breast cancer cells, IGF-I receptor (IGF-IR) function is strongly linked with ER action. IGF-IR and ER are co-expressed in breast tumors and ER-negative breast cancer cells, usually displaying a more aggressive phenotype, often have low levels of IGF-IR and IGF is not mitogenic. Thus, the effect of high level insulin on ER negative breast cancer may be explained, in part, by lower susceptibility regarding IGF function. Due to the insufficient number of cases, further stratification by both the receptor status combined and the menopausal status could not be conducted. The possibility that IGF family members and insulin levels differ with the hormone receptors status should be further pursued in future large-scale studies.

In the present study, breast cancer risk was not associated with levels of IGF-I and IGFBP-3, before or after adjusted for each other. Several recent epidemiological studies pointed to an increased risk of breast cancer in women with comparatively elevated plasma IGF-I levels, expressed either as absolute concentrations, or relative to levels of IGFBP-3 (IGF-I/IGFBP-3 ratio), and the findings appear to hold largely for premenopausal women. Mutual adjustment of IGF-I and IGFBP-3 levels appears to strengthen the association of each of these factors with breast cancer risk (Bruning et al., 1995; Hankinson et al., 1998; Toniolo et al., 2000; Krajcik et al., 2002). Contrary to the results from these previous two cohort studies (Hankinson et al., 1998; Toniolo et al., 2000), as well as from several case-control studies, Kaaks et al. found no global association with results based on large pooled cohorts in Northern and Southern Sweden. Furthermore, they observed an association of breast cancer risk with IGF-I levels in older women, recruited at age 55 or higher, which was not observed earlier (Kaaks et al., 2002). In a case-control study conducted among Chinese women (Yu et al., 2002), a dose-response relationship between plasma IGF-I and breast cancer risk was observed, especially in premenopausal women. These results are consistent with the findings for Caucasian, indicating that IGF-I may play a similar role in breast cancer in different ethnic groups. However, we have failed to detect any positive association between IGF-I and Japanese women breast cancer risk. Because no other epidemiological studies of IGF have so far been performed for Japanese women, we can not conclude whether this discrepancy is because of the study population or the study itself.

A high IGFBP-3 level is generally associated with a reduced risk of cancer. However, two case-control studies, one from Canada (Del Giudice et al., 1998) and the other from China (Yu et al., 2002), suggested that risk was increased in association with relatively high levels. Most of the currently available data are from case-control studies, some nested within prospective investigations. Case-control studies are generally unable to establish the temporal nature of an association due to possible effects of the disease process on blood levels of the molecules of interest and are potentially susceptible to selection and information biases.

In conclusion, the present study suggested that the plasma insulin level is a predictor of postmenopausal breast cancer, particularly in women with a high BMI (>23.07). In addition, we observed a strong relation of insulin with ER negative breast cancer. Additional studies are now needed to clarify the role of glucose metabolism pathways in breast cancer development and interaction of IGF systems in premenopausal versus postmenopausal women.

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References

Bruning PF, Van Doorn J, Gonfere JMG, et al (1995). Insulin-like growth-factor-binding protein 3 is decreased in early-stage operable pre-menopausal breast cancer. *Int J Cancer*, **62**, 266-70.

Cullen KJ, Yee D, Sly WS, et al (1990). Insulin like growth factor receptor expression and function in human breast cancer. *Cancer Res*, **50**, 48-53.

Del Giudice ME, Fantus IG, Ezzat S, et al (1998). Insulin and related factors in premenopausal breast cancer risk. *Breast Cancer Res Treat*, **47**, 111-20.

De Perola G, Cospite MR, Giaculli VA, et al (1993). Insulin-like growth factor I and dehydroepiandrosterone sulphate in obese women. *Int J Obesity*, **17**, 481-3.

Dunn SE, Kari FW, French J, et al (1997). Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res*, **57**, 4667-72.

Hankinson SE, Willett WC, Colditz GA, et al (1998). Circulating concentration of insulin-like growth factor-I and risk of breast cancer. *Lancet*, **351**, 1393-6.

Henderson BE and Feigelson HS (2000). Hormonal carcinogenesis. *Carcinogenesis*, **21**, 427-33.

Hirose K, Tajima K, Hamajima N, et al (1999). Effect of body size on breast-cancer risk among Japanese women. *Int J Cancer*, **80**, 349-55.

Kaaks R, Lundin E, Manjer J, et al (2002). Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in Northern and Southern Sweden. *Cancer Causes Control*, **13**, 307-16.

Kahn BB and Flier JS (2000). Obesity and insulin resistance. *J Clin Invest*, **106**, 473-81.

Kaya SA, Folsom AR, Soler JT, Preneas RJ Potter JD (1991). Association of body mass and fat distribution with sex hormone concentrations in postmenopausal women. *Int J Epidemiol*, **20**, 151-6.

Krajcik RA, Borofsky ND, Massardo S, Orentreich N (2002). Insulin-like growth factor I (IGF-I), IGF-binding proteins, and beast cancer. *Cancer Epidemiol Biomark Prev*, **11**, 1566-73.

Mayer-Davis EJ, Monaco JH, Hoen HM, et al (1997). Dietary fat and insulin sensitivity in a triethnic population; the role of obesity (IRAS). *Am J Clin Nutr*, **65**, 79-87.

Milazzo G, Giorgino F, Damante F, et al (1992). Insulin receptor expression and function in human breast cancer cell lines. *Cancer Res*, **52**, 3924-30.

Osborne CK, Clemmons DR, Arteaga CI (1990). Regulation of breast cancer growth by insulin-like growth factors. *J Steroid Biochem Mol Biol*, **37**, 805-9.

Papa V, Milazzo G, Goldfine ID, Waldman FN, Vigneri R (1997). Sporadic amplification of the insulin receptor gene in human breast cancer. *J Endocrinol Invest*, **20**, 531-6.

Pekonen F, Partanen S, Makinen T, Rutanen EM (1988). Receptors for epidermal growth factor and insulin-like growth factor-I and their relation to steroid receptors in human breast cancer. *Cancer Res*, **48**, 1343-7.

Poretsky L and Kalin MF (1987). The gonadotropic function of insulin. *Endocrinol Rev*, **8**, 132-41.

Shafie SM, Gibson SL, Hilf R (1977). Effect of insulin and estrogen on hormone binding in R3230 AC mammary adenocarcinoma. *Cancer Res*, **37**, 4641-9.

Stoll BA (1998). Western diet, early puberty, and breast cancer risk. *Breast Cancer Res Treat*, **49**, 187-93.

Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ (1996). Dietary fats and insulin action. *Diabetologia*, **39**, 621-31.

Tajima K, Hirose K, Inoue M, et al (2000). A model of practical cancer prevention for out-patients visiting a hospital:the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). *Asian Pacific J Cancer Prev*, **1**, 35-47.

Toniolo P, Bruning PF, Akhmedkhanov A, et al (2000). Serum insulin-like growth factor-I and breast cancer. *Int J Cancer*, **88**, 828-32.

Webster NJG, Resnik JL, Reichart DB, et al (1996). Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression. *Cancer Res*, **56**, 2781-8.

Yoo KY, Tajima K, Miura S, et al (1997). Breast cancer risk factors according to estrogen and progesterone receptor status: a case-control analysis. *Am J Epidemiol*, **146**, 307-14.

Yu H, Jin F, Shu X-O, et al (2002). Insulin-like growth factors and breast cancer in Chinese women. *Cancer Epidemiol Biomark Prev*, **11**, 705-12.