

Relations of Insulin Resistance and Serum Concentrations of Estradiol and Sex Hormone-binding Globulin to Potential Breast Cancer Risk Factors

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There is a hypothesis that hyperinsulinemia or insulin resistance may be a mediator for breast cancer risk factors. On the other hand, some, but not all, of the well-known risk factors of breast cancer have been associated with serum estrogen concentrations. We assessed the relationships of potential breast cancer risk factors to indicators of insulin resistance, fasting plasma insulin concentration and homeostasis model assessment insulin resistance (HOMA-R), in 88 postmenopausal Japanese women. We also examined whether insulin resistance would explain the association of breast cancer risk factors with serum estradiol and sex hormone-binding globulin (SHBG). Information on potential breast cancer risk factors, such as demographic characteristics, smoking and drinking habits, diet, exercise, menstrual and reproductive factors, was obtained by self-administered health questionnaire including a validated semiquantitative food frequency questionnaire. Body mass index (BMI) was significantly correlated with the ratio of estradiol to SHBG (Spearman $r=0.30$, $P=0.0004$), fasting plasma insulin ($r=0.45$) and HOMA-R ($r=0.43$, $P=0.0001$) after controlling for age. The correlations were still significant between BMI and estradiol/SHBG ratio ($r=0.21$, $P=0.047$) after controlling for fasting plasma insulin and between BMI and fasting plasma insulin ($r=0.40$, $P=0.0001$) as well as HOMA-R ($r=0.38$, $P=0.0003$) after controlling for estradiol/SHBG ratio. There is a possibility that effect of BMI on breast cancer risk is mediated by both insulin resistance and estrogen metabolism.

Key words: Breast cancer — Estradiol — Insulin resistance — Obesity — Risk factors

Endogenous hormones, particularly estrogens, have been generally believed to play a central role in the development of breast cancer.¹⁾ Positive association between serum estradiol and breast cancer risk has been confirmed in postmenopausal women.^{2,3)} The known breast cancer risk factors such as age at menarche, nulliparity, and obesity may be mediated by an estrogen mechanism. Therefore, the relationships between estrogen profile and some of the breast cancer risk factors have been investigated, but the results are inconsistent.^{4–8)}

Recent epidemiological studies showed that high serum C-peptide, a peripheral marker of insulin secretion, or plasma fasting insulin level was significantly associated with risk of breast cancer.^{9,10)} Insulin promotes the growth of rat mammary carcinoma cells.¹¹⁾ Hyperinsulinemia may be a significant risk factor for breast cancer. Obesity, a well-known risk factor of postmenopausal breast cancer was accompanied by hyperinsulinemia.^{12,13)} Dietary fat, a suspected breast cancer risk factor, was associated with blood insulin concentration.^{13,14)} Fat intake modified the binding of insulin receptors and the rate of insulin-stimu-

lated glucose transport.¹⁵⁾ These observations led to the hypothesis that hyperinsulinemia or insulin resistance rather than estrogen profile may be a link between breast cancer risk factors and breast cancer.^{16,17)} According to this hypothesis, the previously reported positive association between estradiol concentration and breast cancer risk factors, such as obesity and fat intake, may be explained by the influence of hyperinsulinemia on serum estrogen concentrations.¹⁸⁾ Therefore, we evaluated the relationships between insulin resistance and potential breast cancer risk factors in postmenopausal women and examined whether insulin resistance confounds any observed relations between estrogen status and potential breast cancer risk factors.

MATERIALS AND METHODS

We focused on women who were postmenopausal, defined as cessation of menses for 12 or more months, among the participants in the health check-up program provided by a general hospital in Gifu.¹⁹⁾ A total of 103 postmenopausal women agreed to participate in the present study and completed a self-administered questionnaire between September, 1996 and August, 1997 (the response

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rate was 96.4%). Those who returned the questionnaire with incomplete information were interviewed by a nurse epidemiologist to ascertain complete data.

We asked the participants about potential breast cancer risk factors; marital status, body size, smoking and drinking habits, diet, exercise, and menstrual and reproductive histories. The validated semiquantitative food frequency questionnaire included questions on average frequency of 169 food items during the year prior to the study and the usual serving size of each food item. Individual nutrient intake was estimated from frequency of intake and portion size using the Standard Tables of Food Composition in Japan, 4th ed., published by the Science and Technology Agency of Japan. Detailed information on the questionnaire, including results from validity tests, have been described elsewhere.^{20, 21)}

Exercise was assessed by asking the average hours per week spent performing various kinds of activities during the past year. The details are described elsewhere.²²⁾

A fasting blood sample was obtained from each subject in the morning. The blood was centrifuged, and the serum was separated. Plasma glucose and insulin levels were measured using a glucose-oxidase method and a double antibody radioimmunoassay. The intra-assay coefficients of variation (CV) were 0.7% for glucose and 6.2% for insulin. Serum estradiol and sex hormone-binding globulin (SHBG) were measured by immunoradioassay. Serum estradiol concentration was determined using kits purchased from Diagnostic Products, Chiba, after extraction. Serum SHBG concentration was determined using kit from Pharmacia & Upjohn Co., Ltd., Tokyo. The CV were 10.9% for estradiol and 7.8% for SHBG.

We excluded women who were taking hormone replacement therapy or other hormones ($n=2$), or had a history of breast cancer ($n=3$), ovarian cancer ($n=1$), or metabolic and endocrine diseases such as diabetes mellitus ($n=3$) and thyroid disease ($n=3$). Of the 91 eligible women, 88 had sufficient sera available for hormone assays.

As indicators of insulin resistance, we used fasting plasma insulin concentration and the homeostasis model assessment insulin resistance (HOMA-R). HOMA-R is derived from fasting plasma glucose and insulin data based on the homeostasis model.²³⁾ The relationships between the indices of insulin resistance and potential breast cancer risk factors were determined using Spearman correlation coefficients or ANOVA. Age or age plus the ratio of estradiol to SHBG were included in the models as covariates to calculate the adjusted means or the partial correlation coefficients. The individual nutrient intake was log-transformed and adjusted for total energy using the method proposed by Willett.²⁴⁾ Potential confounding effects of insulin resistance for associations between serum estrogen and SHBG and potential breast cancer risk factors were examined by comparing the magnitudes of

the associations before and after the adjustment for indices of insulin resistance. All statistical analyses were performed using SAS programs.²⁵⁾

RESULTS

The characteristics of the 88 postmenopausal women are presented in Table I.

Weight and body mass index (BMI) were significantly correlated with fasting plasma insulin and HOMA-R after controlling for age (Table II). These correlations were still strong after additional adjustment for the ratio of estradiol to SHBG, which may reflect bioavailable estradiol. Age at menarche was marginally inversely correlated with HOMA-R after controlling for age ($P=0.06$) but the correlation was attenuated after controlling for the ratio of estradiol to SHBG. Number of pregnancies was signifi-

Table I. Selected Characteristics of 88 Postmenopausal Japanese Women

Variable	Mean±SD
Age (yr)	54.2±6.5
Body mass index (kg/m ²)	22.9±2.7
Age at menarche (yr)	13.9±1.7
Age at first birth (yr)	24.6±2.9
Number of births	2.1±0.8
Years since menopause (yr)	6.7±5.3
Exercise (METs ^{a)} hours/week)	14.7±19.6
Alcohol (ml/day)	7.2±13.5
Marital status (%)	
Married	86.4
Separated/divorced	3.4
Widowed	9.1
Never married	1.1
Smoking (%)	
Current	10.2
Past	5.7
Nutrient and food intake per day	
Energy (kcal)	2189±738
Total protein (g)	92.7±34.9
Total fat (g)	62.3±25.4
Animal fat (g)	25.9±14.0
Fish fat (g)	5.0±2.9
Vegetable fat (g)	31.7±11.6
Carbohydrate (g)	299±102
Crude fiber (mg)	6.0±2.9
Vitamin A (IU)	4363±2993
Vitamin C (mg)	176±114
Vitamin E (mg)	9.4±4.0
Carotene (mg)	5598±4165
Soy products (g)	62.3±41.0

a) METs, metabolic equivalents.

Table II. Partial Correlations of Insulin Resistance with Body Size, Exercise, and Menstrual and Reproductive Factors

	Fasting insulin (6.8±4.1 mU/liter) ^{b)}		HOMA-R ^{a)} (1.6±1.3)	
	Age-adjusted	Adjusted ^{c)}	Age-adjusted	Adjusted ^{c)}
Height	-0.08	-0.09	-0.06	-0.07
Weight	0.44 **	0.40 **	0.43 **	0.37 **
Body mass index	0.45 **	0.40 **	0.43 **	0.38 **
Exercise	0.01	-0.05	0.02	-0.04
Age at menarche	-0.17	-0.14	-0.21	-0.18
Age at regular menstrual cycle started	-0.12	-0.11	-0.13	-0.13
Age at first birth	-0.15	-0.12	-0.13	-0.10
No. of pregnancies	0.21	0.22 *	0.14	0.15
No. of births	0.18	0.18	0.15	0.16
Months of lactation	0.05	0.04	-0.01	-0.02
Years since menopause	0.13	0.13	0.14	0.15

a) HOMA-R, homeostasis model assessment.

b) Mean±SD.

c) Adjusted for age and the ratio of estradiol to sex hormone-binding globulin ratio.

* $P < 0.05$, ** $P < 0.01$.

Table III. Partial Correlations of Serum Concentrations of Estradiol and SHBG with Body Size, Exercise, and Menstrual and Reproductive Factors

	Estradiol (20.6±34.8 pg/ml) ^{b)}		SHBG ^{a)} (79.1±32.8 nmol/liter)		Estradiol/SHBG (0.30±0.48)	
	Age-adjusted	Adjusted ^{c)}	Age-adjusted	Adjusted ^{c)}	Age-adjusted	Adjusted ^{c)}
Height	0.003	0.01	-0.03	-0.06	0.02	0.04
Weight	0.09	0.03	-0.34 **	-0.26	0.32 **	0.23 *
Body mass index	0.15	0.10	-0.28 **	-0.18	0.30 **	0.21 *
Exercise	0.18	0.10	-0.11	-0.13	0.15	0.11
Age at menarche	-0.08	-0.06	0.14	0.10	-0.13	-0.09
Age at regular menstrual cycle started	0.06	0.07	0.21 *	0.19	-0.03	-0.002
Age at first birth	0.13	0.16	0.20	0.17	-0.14	-0.10
No. of pregnancies	-0.01	-0.04	-0.01	0.05	-0.02	-0.08
No. of births	0.02	0.06	0.01	0.06	0.002	-0.05
Months of lactation	-0.01	-0.05	-0.06	-0.05	0.02	0.01
Years since menopause	0.002	-0.02	-0.01	0.03	-0.01	-0.05

a) SHBG, sex hormone-binding globulin.

b) Mean±SD.

c) Adjusted for age and fasting insulin concentration.

* $P < 0.05$, ** $P < 0.01$.

cantly correlated with fasting plasma insulin after controlling for the covariates.

The correlations of weight and BMI with the ratio of estradiol to SHBG were attenuated, but still statistically significant, after controlling for plasma insulin (Table III). Significant correlations of serum SHBG with BMI and age

at which regular menstrual cycles started did not remain significant after controlling for plasma insulin, but the changes were not great. The results were almost identical when HOMA-R instead of plasma insulin was controlled.

Smoking status was significantly associated with serum estradiol ($P=0.02$); age-adjusted means of estradiol were

13.1, 11.7, and 29.8 pg/ml in never, current and past-smokers, respectively. Additional adjustment for plasma insulin did not change the result substantially.

None of the nutritional factors studied was significantly correlated with insulin resistance after controlling for age or age plus the ratio of estradiol to SHBG. For example, the correlation coefficient between total fat intake and fasting plasma insulin was -0.05 when either age only or age plus the ratio of estradiol to SHBG was controlled.

DISCUSSION

Age at menarche, age at first birth, parity, and obesity are well known risk factors of breast cancer in postmenopausal women. In the present study, BMI was positively correlated with both indices of insulin resistance and estradiol/SHBG ratio. Each of these correlations, without considering the mutual confounding effects, has been reported previously.^{8, 26, 27} Strong correlations between BMI and indices of insulin resistance after controlling for estradiol/SHBG ratio support the possibility that insulin resistance would be partially responsible for the effect of BMI on breast cancer risk. However, the correlation of BMI with estradiol/SHBG ratio was still strong after the adjustment for index of insulin resistance. Thus, we cannot rule out a possible role of estrogen as a mediator for BMI to affect breast cancer risk. Increased aromatase activity in adipose tissue may also contribute to raise the level of bioavailable estrogen in postmenopausal women, which results in increased breast cancer risk.

We should note that the present study did not assess direct relationships between insulin resistance and estrogen profile and breast cancer, and therefore, we could not clarify whether insulin resistance or estrogen status is a mediator between BMI and breast cancer. Fasting insulin was associated with risk of breast cancer in a study on premenopausal women.¹⁰ Low BMI has been reported as a risk factor of premenopausal breast cancer,²⁸ while a non-significant but positive association between BMI and fasting insulin has been reported in premenopausal women.²⁹ These observations do not support a role of insulin resistance as a mediator between BMI and breast cancer. It is possible that the association between BMI and insulin resistance or estrogen status might be relevant to other diseases, such as endometrial cancer.

It has been postulated that the effect of age at menarche on breast cancer risk may be mediated simply by the relation of early menarche to a long duration of exposure to ovarian steroid.¹ However, we observed moderate inverse associations between age at menarche and indices of insulin resistance. Earlier menarche was associated with earlier onset of hyperinsulinemia in a previous study.³⁰ Persistent hyperinsulinemia after puberty may partially explain the association between age at menarche and breast cancer.

Hyperinsulinemia or insulin resistance did not appear to link the other factors studied to breast cancer. In particular, the observed positive correlations of number of pregnancies with plasma insulin and HOMA-R are inconsistent with the expected protective effect of high parity against breast cancer.

We also failed to find significant associations of estradiol or the ratio of estradiol to SHBG with age at first birth, parity, exercise and diet. The results were similar to those from previous studies in postmenopausal women.^{5, 7, 31, 32} We observed higher estradiol levels in women who had quit smoking, but not in those who continued smoking. The reason for this inconsistency is not clear.

One of the concerns for the present study is the possibility that immunoreactivities which cross-react with the estradiol assay might have affected our findings. The observed associations between the breast cancer risk factors and estradiol or the ratio of estradiol to SHBG may reflect the effects of the breast cancer risk factors on compounds which possess such cross-reactivities.

The cross-sectional nature of the data does not allow causal inference regarding the associations observed. In addition, we expected that interrelationships between estrogen profile, insulin resistance, and breast cancer risk factors might be complicated. Therefore, adjustment for the ratio of estradiol to SHBG or adjustment for insulin resistance would not elucidate the etiologic pathway between these factors and we must be cautious in interpreting the results of multivariate analyses. Among the potential risk factors we examined, however, only BMI was commonly associated with the ratio of estradiol to SHBG and indices of insulin resistance.

The reliance on a single sample may have reduced our ability to detect a significant association. High reproducibility of serum insulin in samples taken one year apart was reported.³³ We could not use more exact methods to evaluate insulin resistance, such as hyperinsulinemic euglycemic clamp and insulin suppression test. However, fasting insulin concentration as well as HOMA-R has been validated against hyperinsulinemic euglycemic clamp in nondiabetic subjects and is considered to be a good surrogate for insulin resistance.^{34, 35}

The study subjects might not be representative of the general population. They are likely to be health conscious and may have previously attended health check-up programs. As measurement of blood glucose but not sex hormones is often included in such programs, some women may have changed their lifestyle or diet after they knew their blood glucose levels. The observed association regarding indices of insulin resistance and breast cancer risk factors might be affected by this bias.

A recent epidemiological study showed that insulin-like growth factor I (IGF-I) was markedly associated with risk

of premenopausal breast cancer.³⁶⁾ We did not include IGF-I measurement. Insulin regulates the serum level of IGF-binding protein-1, one of the key binding proteins of IGF-I.³⁷⁾ Therefore, the associations of potential breast cancer risk factors with indices of insulin resistance might reflect those with IGF-I to some extent. However, IGF-I may be an important mediator of breast cancer. Although we found no association between soy product intake and any of serum hormones and HOMA-R, potential preven-

tive effect of phytoestrogen may be related to suppression of IGF-I production.¹⁷⁾

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