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Serum Levels of Leptin, Insulin, and Lipids in Relation to Breast Cancer in China

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Epidemiological studies have found obesity to be a risk factor for women's breast cancer. The present study was to investigate whether there is a relationship between serum levels of leptin, insulin, and lipids and breast cancer incidence, in order to find experimental evidence that would be helpful in the diagnosis and prevention of breast cancer. Blood samples were collected from 130 patients with mammary disease and 103 healthy control subjects. Serum leptin, insulin, and lipids were determined by radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISA), and Biochemistry Auto–analyzer, respectively. The data analysis was performed by use of the SPSS10.0 computer software. We found that the serum levels of leptin, insulin, and triglyceride (TG) were clearly higher in patients with breast cancer than in patients with benign breast disease and healthy controls, while serum HDL-C levels were lower in breast cancer patients ($p < 0.03$). Moreover, serum leptin levels were significantly correlated with BMI (body mass index) among three groups, whereas serum insulin levels were unrelated to BMI among three groups. Furthermore, the serum levels of leptin and insulin were not associated with menopausal status in patients with mammary disease ($p > 0.05$); however, the serum levels of F-Chol, T-Chol, TG, LDL-C, and APOB were significant higher in postmenopausal cases than those in premenopausal cases ($p < 0.025$). Interestingly, logistic regression analysis showed that subjects with elevated serum levels of leptin, insulin, TG, APOA1, and reduced level of serum HDL-C displayed increased risk of developing breast cancer than those with the normal levels, respectively. In conclusion, the present study suggested that aberrant serum levels of leptin, insulin, and lipids might play an important role in carcinogenesis of breast cancer. The elevated serum levels of leptin, insulin, TG, APOA1, and reduced level of serum HDL-C may be correlated with increased risk of breast cancer, suggesting that one way of preventing breast cancer would be carried out by controlling the intake of food.

Key Words: Breast cancer; leptin; insulin; lipids; serum levels.

Introduction

Breast cancer is the most common cancer in women and the main cause of death from cancer in the world *(1)*. The incidence of breast cancer is increasing in China due to the improvement of people's quality of life and changes in the people's lifestyle *(2)*.

Many epidemiological studies have found that obesity might be associated with increased risk of breast cancer in postmenopausal women. Among the middle-aged and old-aged women of the United States and other Western countries, weight increment has been considered a risk factor of increased breast cancer *(3,4)*. Therefore, it is important for diagnosis and prevention of breast cancer to elucidate the relationship between obesity-related biomarkers and mammary diseases.

Of these biomarkers, leptin was originally described in the mouse as the product of the obese gene, which was shown to encode a hormone and express predominantly in adipose tissues. It has been demonstrated that plasma leptin correlates to body fat content and a several types of cancers. Hardwick et al. pointed out that leptin is a growth factor in colonic epithelial cells *(5)*. Stattin et al. also suggested that leptin is a risk factor for colon cancer, and that leptin may provide a link between obesity and colon cancer *(6)*. Somasundar et al. demonstrated that leptin acts as a growth factor for prostate cancer cells in vitro *(7)* and prostate cancer cell migration was enhanced by leptin and inhibited 50–70% with the addition of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) inhibitors *(8)*. Serum levels of leptin have been found to be elevated in patients with prostate cancer *(9)*. O'Brien et al. reported that leptin was expressed in malignant epithelial cells of the breast *(4)*.

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Table 1
Serum Levels of Leptin and Other Biomarkers in Patients with Breast Diseases and in Healthy Controls

	Breast cancer cases (n = 90)		Breast benign disease (n = 40)		Healthy controls (n = 103)	
Variable	Mean	SD	Mean	SD	Mean	SD
Age, y	45.88	9.20	49.46	10.51	46.58	9.60
BMI, kg/m^2[a,b]	25.05	3.55	22.46	2.71	23.36	3.06
Leptin, μg/L[a,b]	13.57	0.66	10.87	1.09	9.46	0.60
Insulin,mU/L[a,b]	12.29	14.62	6.57	6.93	5.34	4.42
TG, mmol/L[a,b]	1.58	1.16	1.09	0.68	1.16	0.60
F-Chol, mmol/L	1.83	0.58	1.68	0.52	1.69	0.50
T-Chol, mmol/L	4.14	0.85	4.06	0.93	4.25	1.07
HDL-C, mmol/L[a,b]	1.14	0.29	1.31	0.34	1.37	0.34
LDL-C, mmol/L	2.69	0.82	2.54	0.89	2.64	0.94
APOA1, mmol/L	1.38	0.31	1.43	0.32	1.44	0.27
APOB, mmol/L	0.69	0.20	0.62	0.19	0.70	0.24
APOA1/APOB	2.19	0.89	2.50	0.98	2.30	1.04
% Smokers	2.22%	2.50%	2.91%			
% Alcohol users	5.60%	5.00%	4.85%			

[a]Comparison between breast cancer patients and benign disease patients, and healthy controls, $p < 0.03$.
[b]Comparison was made between benign breast disease patients and healthy controls, $p > 0.05$.

Leptin also interacts with other endocrine factors, including insulin. Increased body fat content is accompanied by low insulin sensitivity, which is compensated with increased insulin secretion *(10,11)*. Segal et al. demonstrated that insulin resistance could be associated with elevated plasma leptin levels independent of body fat mass *(12)*. Stattin et al. found only weak support for an association of insulin with colon cancer *(9)*.

Taken together, these findings provided us with a biological explanation for the observed associations between obesity, physical activity, and cancer. Although much is known about the potential of leptin and insulin as regulators of body fat content *(13,14)*, little is clear about the serum levels of leptin, insulin, and especially for lipids, in connection with breast cancer. In the current study, we explored the relationship between serum levels of leptin, insulin, and lipids and tumorigenesis of breast cancer in 130 patients with mammary disease and 103 healthy control subjects.

Results

Serum Levels of Leptin, Insulin, and Lipids in Patients with Breast Diseases and Controls

Table 1 illustrates the mean levels of serum leptin, insulin, and lipids in patients with breast diseases and in controls. It demonstrates that the serum levels of leptin, insulin, and TG were significantly higher in patients with breast cancer than in patients with benign breast disease and healthy controls, while serum HDL-C levels were lower in breast cancer patients ($p < 0.03$). However, such differences were not found between benign disease patients and controls ($p > 0.05$). These results have been adjusted with the BMI and ages because both weight and age appeared to be somewhat different. Moreover, Fig. 1 showed that serum leptin levels were significantly associated with BMI among three groups ($r = 0.327$, $p < 0.001$; $r = 0.416$, $p < 0.001$, and $r = 0.525$, $p < 0.001$, respectively), whereas serum insulin levels were unrelated to BMI among three groups ($r = 0.164$, $p > 0.05$; $r = 0.054$, $p > 0.05$, and $r = 0.168$, $p > 0.05$, respectively). With respect to BMI, smoking, and alcohol intake, there was statistically no difference among the three groups ($p > 0.05$).

Relationship between Serum Levels of Leptin and Other Biomarkers and Breast Diseases According to Menopausal Status

The serum levels of leptin and insulin were not associated with menopausal status in patients with mammary disease ($p > 0.05$); however, the serum levels of F-Chol, T-Chol, TG, LDL-C, and APOB were significant higher in postmenopausal cases than those in premenopausal cases, respectively ($p < 0.025$). The data were summarized in Table 2.

Multiple Regression Analysis

Table 3 illustrates the association between risk of breast cancer and serum levels of leptin, insulin, and lipids. It shows that subjects with elevated serum levels of leptin, insulin, TG, APOA1, and reduced level of serum HDL-C displayed increased risk of developing breast cancer than those subjects with the normal levels, respectively. Their corresponding odd ratios (ORs) were 1.140 (95% CI: 1.076–1.209), 1.110 (95% CI: 1.017–1.209), 1.879 (95% CI: 1.148–3.076), 5.726 (95% CI: 1.238–26.480), and 0.035 (95% CI: 0.007–0.162).

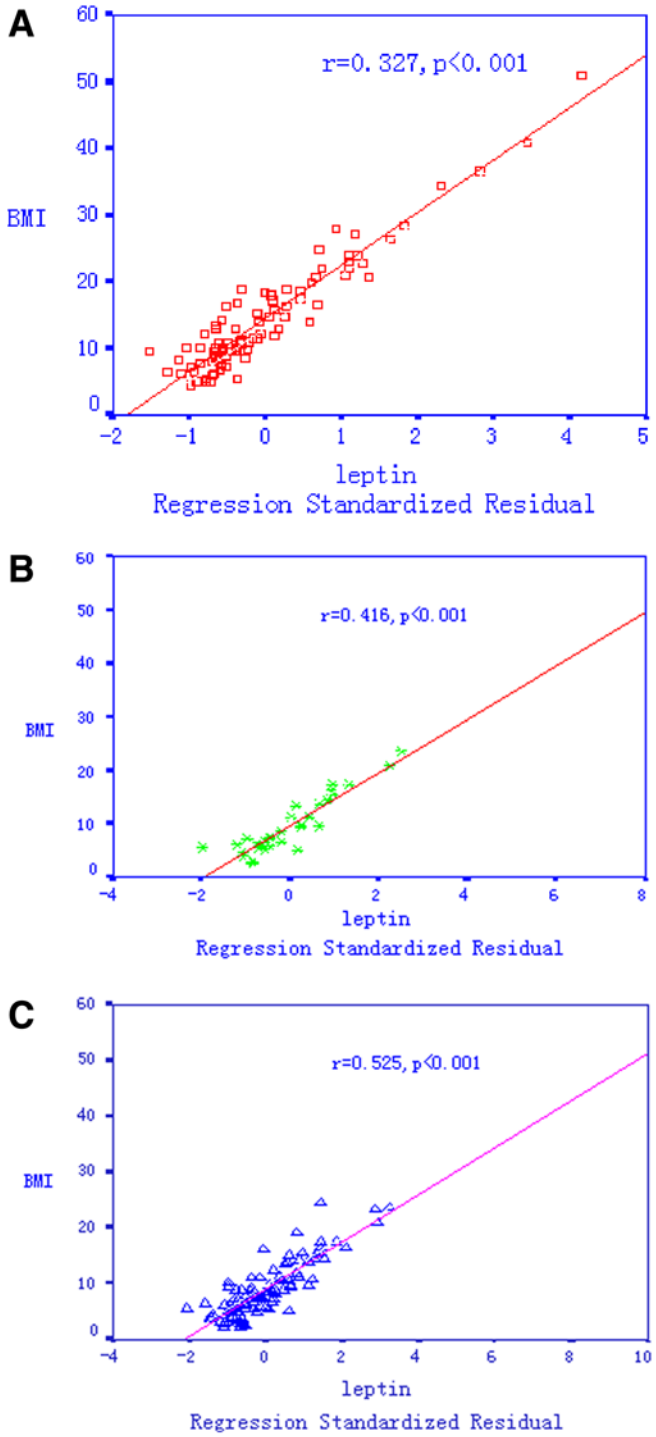

Fig. 1. Schematic correlation between serum level of leptin and BMI by the scatter plots and linear regression among three groups: **A**, in breast cancer group; **B**, in breast benign disease group; **C**, in healthy controls group.

Discussion

Compelling evidence has suggested that obesity could be a risk factor for women's breast cancer, and several factors affecting weight have been found to contribute to tumorigenesis of many types of cancers, including breast cancer. To further evaluate the roles of some biomarkers' alterations regarding obesity in breast cancer, we used RIA, ELISA, and Biochemistry Auto-analyzer to examine the serum levels in 130 patients with malignant and benign breast diseases.

The present study provided experimental evidence for diagnosis and prevention of breast cancer in three aspects. First, we found significant elevation of serum levels of leptin, insulin, and TG in 90 breast carcinomas compared with those in 40 benign cases and 103 controls. Our results were consistent with the findings reported previously by Tessitore et al. *(3)* and different from other studies that did not show any association between leptin and malignant breast tumor *(15,16)*. While obesity is a known risk factor for women's breast cancer, the molecular mechanisms involved are unclear *(16)*. Much attention was drawn to leptin, the product of the obese gene in breast neoplasia. For instance, Hu et al. reported that leptin enhanced anchorage-dependent proliferation by 138% in human breast carcinoma–derived T-47D cells and 50% in human breast epithelial HBL100 cells *(17)*. Our findings would be consistent with that conclusion and provide a biological explanation for the observed relationship between obesity, physical activity, and breast cancer. Considering that the body mass could be associated with the development of breast cancer *(18,19)*, we adjusted these results obtained with the BMI.

Besides leptin, insulin has been found to correlate to breast cancer. Del Giudice et al. and Hirose et al. suggested that insulin is also a risk factor for the breast cancer *(20,21)*. We found that the serum levels of insulin were clearly higher in patients with breast cancer than those in patients with breast disease benign and controls. The mechanism underlying insulin-induced breast cancer involves insulin-like growth factor (IGF-I) systems, and insulin decreases IGFBP production and secretion, thereby increases the bioavailability of IGF-I *(22)*. Epidemiological evidence indicated that an increased ratio of IGF-I to IGFBP in the circulation was associated with an increased risk for the development of breast cancer *(23,24)*.

Second, we explored the relationship between serum levels of leptin and other biomarkers and breast diseases in premenopausal and postmenopausal women. Our results indicated that the serum levels of leptin and insulin were not associated with menopausal status in patients with mammary disease. In contrast, a positive association between the serum levels of leptin and insulin and menopausal status was found in breast carcinomas *(25,26)*. Del Giudice et al. reported that circulating insulin levels were elevated in premenopausal women with breast cancer *(20)*. Hirose et al. suggested that the plasma level of insulin is a predictor of breast cancer in postmenopausal obese women *(21)*. There seem to be a complex mechanism responsible for association between menopausal status and insulin in breast carcinomas. In addition, we found that the serum levels of F-Chol, T-Chol, TG, LDL-C, and APOB were significant higher in postmenopausal cases than those in premenopausal cases, respectively. Little is known about the role of lipids in connection with breast

Table 2
Relationship between Serum Levels of Leptin and Other Biomarkers and Breast Diseases According to Menopausal Status

	Premenopause subjects			Postmenopause subjects			
Variable	*n*	Mean	SD	*n*	Mean	SD	*p* value
Leptin, μg/L							
BCP	52	14.61	9.27	38	14.33	7.02	0.879
HC	70	8.23	4.50	33	10.83	5.54	<0.024
BBDP	27	9.49	5.23	13	10.86	6.61	0.608
Insulin, mU/L							
BCP	52	13.54	16.86	38	10.58	10.83	0.346
HC	70	5.33	5.0	33	5.37	2.87	0.964
BBDP	27	6.54	7.30	13	6.71	5.05	0.962
BMI, kg/m^3							
BCP	52	24.91	3.81	38	25.25	3.21	0.658
HC	70	22.96	2.75	33	24.19	3.52	0.057
BBDP	27	22.31	2.77	13	23.27	2.50	0.475
F-Chol, mmol/L							
BCP	52	1.72	0.59	38	2.00	0.51	<0.021
HC	70	1.58	0.49	33	1.92	0.45	<0.010
BBDP	27	1.02	0.54	13	1.84	0.38	0.452
T-Chol, mmol/L							
BCP	52	3.85	0.66	38	4.54	0.94	<0.001
HC	70	3.97	0.95	33	4.83	1.07	<0.001
BBDP	27	3.95	0.89	13	4.67	1.01	0.116
TG, mmol/L							
BCP	52	1.34	0.89	38	1.89	1.40	<0.025
HC	70	1.05	0.56	33	1.39	0.62	0.060
BBDP	27	1.02	0.67	13	1.51	0.66	0.141
HDL-C							
BCP	52	1.19	0.31	38	1.09	0.27	0.104
HC	70	1.34	0.35	33	1.43	0.32	0.246
BBDP	27	1.33	0.36	13	1.18	0.21	0.367
LDL-C							
BCP	52	2.39	0.65	38	3.10	0.86	<0.001
HC	70	2.42	0.85	33	3.12	0.96	<0.001
BBDP	27	2.42	0.87	13	3.19	0.83	0.075
APOAl							
BCP	52	1.34	0.26	38	1.45	0.37	0.123
HC	70	1.41	0.29	33	1.51	0.23	0.077
BBDP	27	1.45	0.34	13	1.31	0.20	0.383
APOB							
BCP	52	0.63	0.18	38	0.78	0.20	<0.001
HC	70	0.65	0.22	33	0.81	0.26	<0.010
BBDP	27	0.59	0.17	13	0.74	0.28	0.140

BCP, Breast cancer patients; HC, healthy controls; BBDP, breast benign disease patients.

cancer, although the lipids substance has been found to correlate with cardiovascular disease risk *(27,28)*.

Third and interestingly, logistic regression analysis showed that subjects with elevated serum levels of leptin, insulin, TG, APOA1, and reduced level of serum HDL-C displayed increased risk of developing breast cancer than those with the normal levels, respectively. Especially, subjects with elevated serum level of APOA1 present more than fivefold increased risk of breast caner than the normal level. This case-control analysis suggested that it would be helpful for the diagnosis and/or prevention of breast cancer to evaluate the risk by combining APOA1 and HDL-C.

In conclusion, the present study suggested that aberrant serum levels of leptin, insulin, and lipids might play an important role in carcinogenesis of breast cancer and be associated with increased risk of breast cancer.

Table 3
Association between Risk of Breast Cancer and Serum Levels of Leptin, Insulin, and Lipids Based on a Case and Control Analysis

Variable		OR[a]	95% CI	*p*
Leptin	0.131	1.140	1.076–1.209	0.001
Insulin	0.103	1.110	1.017–1.209	0.02
TG	0.631	1.879	1.148–3.076	0.012
HDL-C	–3.357	0.035	0.007–0.162	0.001
APOAl	1.745	5.726	1.238–26.480	0.026

[a]ORs and 95% CIs were calculated in a logistic regression model with healthy control as reference group.

Materials and Methods

Specimens

Blood samples were collected after informed consent from 130 female patients who were in hospital for mammary disease in Shanxi Cancer Hospital in Taiyuan, China. The 130 specimens include histologically defined infiltrative ductal carcinoma (n = 61), infiltrative lobular carcinoma (n = 13), medullary carcinoma (n = 6), adenocarcinoma (n = 6), papillary carcinoma (n = 2), tubular carcinoma (n = 1), mucinous adenocarcinoma (n = 1), and breast benign disease (n = 40) specimens. None of these patients had received either radiotherapy or chemotherapy before sampling. Besides the case samples, a total of 103 blood samples were also collected from subjects with no history of cancer and diabetic, adiposity, or endocrine disorders as controls. These controls were randomly selected from the same geographic region and their ages were in the similar range as the cases.

A standardized questionnaire was carried out to collect data concerning age, occupation, weight, and marriage history. Other information, including obstetric and gynecological history, and family history of breast cancer and so on, was obtained from all the subjects.

Sample Collection

All blood samples were drawn from the elbow vein under the same conditions. Before the subjects were sampled, they were prescribed an overnight fast. Sera were obtained from blood samples by processing of clotting and centrifugation. Serum samples can be stored at 2–8°C for 24 h, and could be stored at −20°C for a longer time until analyzes. Serum samples should not be frozen and thawed repeatedly.

Measurement Methods

Leptin level was determined according to radioimmunoassay kit purchased from Linco Research, USA. Measurement of serum insulin was carried out using ELISA kit (Mercodia AB, Sweden). Lipid substances were measured using kit (Randox Laboratories Ltd, UK) on Biochemistry Autoanalyzer (AU600, Olympus, Japan). Detailed procedures are described in the corresponding commercial kits. All specimens were determined in duplicate.

BMI (body mass index) is defined as weight in kilograms divided by the square of height in meters [weight (kg)/height (m^2)]. Obesity status defined by measured BMI is hereafter referred to as obese by measured BMI.

Statistical Analysis

Correlations between the serum levels of leptin, insulin, lipid substance, and BMI in cases and those in controls were evaluated by means of ANOVA. Differences were taken to be significant at $p < 0.05$. The association between risk of breast cancer and serum levels of leptin, insulin and lipids was determined as odd ratio (OR) based on the multiple logistic regression analysis. All data analysis was conducted with SPSS10.0 computer software.

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