Fasting Insulin and Outcome in Early-Stage Breast Cancer: Results of a Prospective Cohort Study

By Pamela J. Goodwin, Marguerite Ennis, Kathleen I. Pritchard, Maureen E. Trudeau, Jarley Koo, Yolanda Madarnas, Warren Hartwick, Barry Hoffman, and Nicky Hood

Purpose: **Insulin, a member of a family of growth factors that includes insulin-like growth factor (IGF)-I and IGF-II, exerts mitogenic effects on normal and malignant breast epithelial cells, acting via insulin and IGF-I receptors. Because of this and because of its recognized association with obesity, an adverse prognostic factor in breast cancer, we examined the prognostic associations of insulin in early-stage breast cancer.**

Patients and Methods: **A cohort of 512 women without known diabetes, who had early-stage (T1 to T3, N0 to N1, and M0) breast cancer, was assembled and observed prospectively. Information on traditional prognostic factors and body size was collected, and fasting blood was obtained.**

Results: **Fasting insulin was associated with distant recurrence and death; the hazard ratios and 95% confidence intervals (CI) for those in the highest (> 51.9 pmol/L) versus the lowest (< 27.0 pmol/L) insulin quartile were 2.0 (95% CI, 1.2 to 3.3) and 3.1 (95% CI, 1.7 to 5.7), respectively. There was some evidence to suggest that the association of insulin with breast cancer outcomes may be nonlinear. Insulin was correlated with body mass index (Spearman r = 0.59, *P* < .001), which, in turn, was associated with distant recurrence and death (*P* < .001). In multivariate analyses that included fasting insulin and available tumor- and treatment-related variables, adjusted hazard ratios for the upper versus lower insulin quartile were 2.1 (95% CI, 1.2 to 3.6) and 3.3 (95% CI, 1.5 to 7.0) for distant recurrence and death, respectively.**

Conclusion: **Fasting insulin level is associated with outcome in women with early breast cancer. High levels of fasting insulin identify women with poor outcomes in whom more effective treatment strategies should be explored.**

J Clin Oncol 20:42-51. © ***2001 by American Society of Clinical Oncology.***

INSULIN IS A member of a family of growth factors that includes insulin-like growth factors (IGF)-I and IGF-II.[1,2] Although insulin is most widely known for its metabolic effects,[3,4] it also has important mitogenic effects, and there is a growing body of evidence that these mitogenic effects are relevant in breast cancer.[5,6] Some mitogenic effects are mediated by the reaction of insulin with the IGF-I receptor, however, insulin stimulation of its own receptor induces transformation in normal breast epithelial cells and promotes proliferation of malignant breast cells in vitro. Overexpression of insulin receptors on breast cancer cells may convey a favorable prognosis,[7] possibly reflecting upregulation of receptors or better differentiation of tumors expressing insulin receptors. We have recently reported higher levels of fasting insulin to be associated with risk of breast cancer development,[8] building on previous work by Bruning et al[9] that identified elevated levels of C-peptide in women with breast cancer. However, the relationship of circulating insulin to breast cancer outcomes has not been studied.

A growing number of reports collectively suggest that obesity is associated with poorer outcomes in women with breast cancer.[10-29] In the study reported here, we sought to confirm this observation in a prospective fashion, and because of the known association of obesity with higher levels of insulin,[3,4] we hypothesized that high levels of insulin would be associated with recurrence and death in women with newly diagnosed breast cancer.

PATIENTS AND METHODS

Population Assembly

A consecutive cohort of women who underwent treatment for operable breast cancer at three University of Toronto hospitals (Mount Sinai Hospital, Women's College Hospital, and St Michael's Hospital) was assembled prospectively between July 1989 and June 1996. Before mid-June 1992, only premenopausal women were recruited (118 in total); these women participated in our study of breast cancer risk.[8] When the study was expanded to examine prognosis, we sought to increase generalizability by recruiting both pre- and postmenopausal women. A logbook was maintained to record eligibility and refusals.

Women were included if they met the following criteria: (1) age less than 75 years and (2) complete resection (lumpectomy with margins clear of invasive cancer or mastectomy) and (3) axillary node dissec-

From the Department of Medicine, Department of Pathology and Laboratory Medicine, and Department of Surgery, Division of Clinical Epidemiology at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital; Toronto-Sunnybrook Regional Cancer Centre; Sunnybrook and Women's College Health Sciences Centre; St Michael's Hospital; and University of Toronto, Toronto, Ontario, Canada.

Submitted November 13, 2000; accepted July 17, 2001.

Supported by the Canadian Breast Cancer Research Initiative, Toronto, and the Medical Research Council of Canada (currently Canadian Institutes of Health Research), Ottawa, Canada.

Address reprint requests Pamela J. Goodwin, MD, Mount Sinai Hospital, 600 University Ave, Ste 1284, Toronto, Ontario M5G 1X5, Canada; email: pgoodwin@mtsinai.on.ca.

0732-183X/01/2001-42/$20.00

tion for previously untreated T1 to T3, N0 to N1, M0 breast cancer. Women were excluded if they met any of the following criteria: (1) prior malignancy (except nonmelanoma carcinoma of the skin or carcinoma-in-situ of the cervix); (2) serious coexisting medical condition, including diabetes type I or II (fasting glucose was not measured); (3) use of medications that could influence key study variables (diet and lipids); (4) inability to speak English; or (5) refusal to provide informed consent. All participants provided written informed consent in accordance with approval granted by the Human Subjects Committee of the University of Toronto.

Measurement

Women underwent the following baseline measurements between 4 and 12 weeks postoperatively, before adjuvant therapy. During a standardized interview, they provided information on demographics, risk factors, and physical activity. Weight was measured using a balance beam scale, after a 12-hour overnight fast, with the woman clothed in a hospital gown. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Fasting blood was collected into tubes containing EDTA anticoagulant, centrifuged immediately, and the plasma stored at minus 70°C. Women completed the Block Food Frequency Questionnaire,[30] recording food intake during the previous year, and they completed a series of psychosocial and eating behavior questionnaires.

Pathology reports were reviewed prospectively to ensure eligibility; pathologic characteristics of the tumors (including hormone receptors) were subsequently abstracted from these reports onto standard forms by one of the authors (Y.M.). Hormone receptors were measured at participating institutions using protein binding or immunohistochemical assays according to the standard practice at each institution.

Insulin was measured on the automated Beckman Coulter Access Immunoassay System (Beckman Coulter Canada Inc, Mississauga, Canada) using the manufacturer's two-epitope immunometric chemiluminescent method. The interassay coefficient of variation was less than 6%. IGF-I, IGF-II, and IGF binding protein-3 (IGFBP-3) were assayed without extraction using the enzyme-linked immunosorbent assay kits marketed by Diagnostic Systems Laboratories (DSL-10 to 2800, DSL-1 to 2600, and DSL-10 to 6600, respectively). Interassay imprecision was of the order of 6% to 8% over the range of values encountered in the study specimens. Cross-reactivity was negligible among insulin, IGF-I, and IGF-II with these selected assay methods. Estradiol was measured using a solid phase competitive chemiluminescent immunoassay carried out on the automated immunoassay analyzer, the Immulite 2000 Immunoassay Analyser (Diagnostic Products Corp, Los Angeles, CA). Laboratory personnel performing these assays were blinded to the clinical status of study participants.

Follow-Up

Participants were observed prospectively, and information on disease recurrence and death was abstracted from medical records and coded in a standard fashion. Eight women were lost to follow-up and were censored at last contact; two of these had experienced a recurrence. Recurrences were categorized using criteria established by the National Cancer Institute of Canada Clinical Trials Group. Research personnel performing follow-up and classifying recurrences were blinded to results of insulin, IGF-I, IGF-II, and IGFBP-3 assays. Analyses were performed as planned when sufficient recurrences had occurred to identify a 1.8-fold increased risk of distant recurrence in obese women, with 80% power and a type I error of 0.05 (two-sided).

Statistical Analysis

Descriptive means, SDs, and/or distributions were generated for all study variables. Distributions of continuous variables were checked for skewness and outliers. This led to the use of the following transformations in statistical analyses, which improved variable attributes and greatly reduced the influence of outliers: insulin (raised to power −0.25); IGF-I, IGF-II, IGFBP-3, and tumor size (square root); BMI (inverse); and estradiol (log). These transformations were reversed for presentation of data. Spearman rank correlation coefficients were calculated to examine correlations. One-way analyses of variance were used to examine the relationship of insulin to pathologic prognostic factors.

Univariate prognostic analyses were performed using the Cox proportional hazards model to examine effects of insulin, IGF-I, IGF-II, estradiol, BMI, traditional prognostic factors (tumor size expressed as T stage, nodal stage, tumor grade, nuclear grade, lymphatic invasion, estrogen receptor [ER], progesterone receptor [PgR]), age, and menopausal status on distant disease-free survival (DDFS) and overall survival (OS). Distant recurrences included those beyond the ipsilateral breast and axilla. The assumption of proportional hazards was checked for each model via smoothed plots of time-dependent coefficients estimated from scaled Schoenfeld residuals.

For the purposes of presentation of prognostic effect sizes, unadjusted hazard ratios and 95% confidence intervals (CI) of distant recurrence and death for continuous variables (insulin, BMI, IGF-I, IGF-II, and estradiol), as well as for recognized categories of traditional prognostic factors and treatment, were calculated using coefficients generated by Cox models. For continuous variables, the 12.5, 37.5, 62.5, and 87.5th percentiles are used as midpoints to represent the four quartile groups of the variable. We then recalculated hazard ratios for insulin after adjustment for each of these traditional prognostic factors and treatment, in turn, recognizing that many were significantly related to insulin levels. The contribution of interaction terms was examined; none was significant. However, because of limited power to detect interactions, some potential interactions (eg, insulin and BMI) were further explored.

Exploratory Cox multivariate analyses were then performed including the following variables: age at diagnosis, tumor stage (T1, T2, or T3), nodal stage (N0 or N1), tumor grade (1, 2, or 3), hormone receptor status (either ER or PgR positive or equivocal or both negative), adjuvant chemotherapy (yes or no), and adjuvant tamoxifen (yes or no). Nuclear grade and lymphatic invasion were not included because they were missing in a nonrandom fashion in a large number of subjects (they were more likely to be missing in women with small, good prognosis tumors who did not receive systemic adjuvant treatment). In Cox models, likelihood ratio test *P* values are reported for whole models and Wald test *P* values for individual variables (for univariate models they agreed very well). All *P* values are two-tailed.

RESULTS

Study Population

A total of 910 women were potential candidates for the study; 152 (16.7%) refused participation. Exclusions were as follows: prior cancer (n = 38, including 30 women with prior breast cancer), serious underlying medical condition (n = 34) or conflicting medication (n = 44) (these two criteria included 35 women with known type I or II diabetes), inability to speak English (n = 74), and other

(n = 33, notably attendance at a participating center for a second opinion without receiving primary cancer treatment at that center). Of the 535 eligible and consenting women, fasting blood was available on 512. Reasons for unavailability in the remaining 23 women were failure to provide a specimen (n = 20) and loss during storage (n = 3). Eight of the 512 available specimens were hemolyzed; insulin was not measured in these samples.

Clinical, treatment, and tumor-related characteristics of the study population are listed in Table 1.[31-33] Median follow-up (censoring patients at death) was 50 months (range, 36 to 112 months). Seventy-six women experienced distant recurrences, and 45 women died. All but three deaths were caused by breast cancer. One woman died of complications of adjuvant chemotherapy, one of promyelocytic leukemia, and one in an accidental fall. None of these women had recurrent breast cancer.

Insulin, BMI, and Related Factors

In our sample, insulin levels ranged from 8 to 340 pmol/L. The distribution of insulin was skewed, 75% of values being below 51 pmol/L, and 95% were below 106 pmol/L. Fasting levels of insulin, IGF-I, IGF-II, IGFBP-3, estradiol, and their correlations are listed in Table 2. Insulin levels were strongly correlated with BMI (Spearman correlation coefficient = 0.59). Although many of the other correlations were statistically significant (because of the large number of women studied), apart from the correlations of IGFBP-3 with IGF-I and IGF-II (reflecting the fact that IGFBP-3 is the major carrier protein for these substances in the circulation), the magnitude of these associations was weak ($r < 0.20$).

Insulin levels were not correlated with time elapsed from surgery for breast cancer (Spearman $r = -0.04$, $P = .34$). There was no evidence they were associated with psychologic distress measured using either the Profile of Mood States[34] (correlation of insulin with total mood disturbance, Spearman $r = 0.05$, $P = .38$) or the Impact of Events Scale[35] (correlation of insulin with total score, Spearman $r = 0.07$, $P = .20$).

Insulin levels were significantly related to tumor stage (T1, 40.7 ± 25.7 pmol/L; T2, 51.6 ± 39.2 pmol/L; T3, 47.7 ± 36.0 pmol/L; $P < .001$), nodal stage (N0, 41.9 ± 25.2 pmol/L; N1, 50.7 ± 41.1 pmol/L; $P = .02$), and tumor grade (grade 1, 39.9 ± 26.7 pmol/L; grade 2, 43.4 ± 33.6 pmol/L; grade 3, 48.7 ± 30.0 pmol/L; $P = .002$). Insulin levels were not significantly related to nuclear grade, lymphatic invasion, ER, or PgR (all $P > .10$). IGF-I was not significantly associated with any prognostic factor, whereas IGF-II levels were significantly higher in women whose tumors were ER negative (ER positive, 579.0 ± 108.3 ng/L; ER negative,

Table 1. Clinical, Treatment, and Tumor-Related Characteristics of the Study Population (N = 512)

Characteristic	No. of Patients	%
Age, years		
Mean ± SD	50.4 ± 9.7	
Range	26-74.4	
Weight, kg		
Mean ± SD	66.8 ± 13.6	
Range	36.4-135.0	
Height, cm		
Mean ± SD	162.0 ± 6.8	
Range	142-182	
BMI, kg/m^2		
Mean ± SD	25.5 ± 5.0	
Range	16.3-54.8	
Menopausal status*		
Premenopausal	289	56.4
Perimenopausal	26	5.1
Postmenopausal	197	38.5
Surgical treatment		
Mastectomy	113	22.1
Lumpectomy	399	77.9
Systemic treatment		
Chemotherapy only†	147	28.7
Chemotherapy plus tamoxifen†	46	9.0
Tamoxifen only	151	29.5
None	168	32.8
Tumor stage		
T1, ≤ 2 cm	288	56.3
T2, 2-5 cm	164	32.0
T3, > 5 cm	24	4.7
TX‡	36	7.0
Nodal stage		
N0	356	69.5
N1	156	30.5
ER		
Positive	314	61.3
Equivocal	28	5.5
Negative	98	19.1
Unknown	72	14.1
PgR		
Positive	285	55.7
Equivocal	29	5.7
Negative	119	23.2
Unknown	79	15.4
Overall tumor grade		
1	73	14.3
2	202	39.5
3	173	33.8
Unknown	64	12.5
Nuclear grade		
1	27	5.3
2	189	36.9
3	144	28.1
Unknown	152	29.7
Lymphovascular invasion		
Present	111	21.7
Absent	262	51.2
Unknown	139	27.1

*Premenopausal (regular menses), perimenopausal (menses not regular but at least one period in past year), postmenopausal (no menses for over a year).

†Fifty patients received anthracycline-based chemotherapy (37 received cyclophosphamide, epirubicin, and fluorouracil,[31] 13 received doxorubicin and cyclophosphamide[32]), and 143 received cyclophosphamide, methotrexate, and fluorouracil.[33]

‡TX = tumor stage unknown, but not T4.

Table 2. Fasting Insulin, IGF-I, IGF-II, and Estradiol

	Level		Spearman Correlations Between Factors			
	Mean	SD	BMI (r)	Insulin (r)	IGF-I (r)	IGF-II (r)
BMI, kg/m^2	25.5	5.0	–	0.59*	−0.19*	0.11*
Insulin, pmol/L	44.6	31.1	0.59*	–	0.04	0.15*
IGF-I, ngm/L	157.8	54.1	−0.19*	0.04	–	0.20*
IGF-II, ngm/L	587.5	108	0.11*	0.15*	0.20*	–
IGFBP-3, ngm/mL	3802.0	833	0.08	0.18*	0.41*	0.50*
Estradiol, pmol/L	201.3	264	−0.19*†	−0.18*	0.37*	−0.25*

*Spearman correlation coefficient significantly different from zero, $P < .05$.
†$r = 0.11$, $P = .13$ in postmenopausal women.

606.8 ± 105.0 ng/L; $P = .02$). BMI was significantly associated with tumor stage (T1, 24.8 ± 4.2 kg/m^2; T2, 26.5 ± 5.4 kg/m^2; T3 28.4 ± 7.8 kg/m^2; $P = .001$), but not with any other prognostic factor.

Univariate Prognostic Associations

Prognostic associations of investigational key variables are listed in Table 3. Fasting insulin was significantly associated with both DDFS and OS (Fig 1), women in the highest quartile of insulin having a two-fold (95% CI, 1.2 to 3.3) increased risk of distant recurrence and an 3.1-fold (95% CI, 1.7 to 5.7) increased risk of death compared with those in the lowest quartile. Age-adjusted hazard ratios were 2.3 (95% CI, 1.4 to 3.8) and 3.4 (95% CI, 1.8 to 6.4), respectively. Insulin seemed to be associated with outcomes in both premenopausal women (hazard ratios for distant recurrence and death were 1.7 [95% CI, 0.9 to 3.1] and 2.7 [95% CI, 1.3 to 5.8], respectively) and postmenopausal women (hazard ratios for distant recurrence and death were 3.7 [95% CI, 1.4 to 9.8] and 5.6 [95% CI, 1.6 to 19.6], respectively), although 95% CI's are broad, reflecting reduced power in these subsets. No significant prognostic effects were seen for IGF-I, IGF-II (before or after adjustment for IGFBP-3 levels), or estradiol. Results were similar when the three women who died without breast cancer recurrence were excluded from the analysis.

As can be seen in Fig 1, the DDFS and OS curves representing the first three quartiles of insulin do not seem to be well separated. This may be a result of the relatively small differences in insulin levels between these quartiles, which are reflected in small differences in hazard ratios. However, it is also possible that a simple linear increase in log hazard is not the best way to model the relationship, especially for the extremes of the insulin range. We investigated the presence of a quadratic relationship as well as a number of thresholds and bending points in an exploratory fashion using bootstrap methods to determine whether the presence of a prognostic effect of insulin was dependent on the functional form of the model used. Addition of a quadratic term did not enhance the explanatory power of our linear model ($P = .30$ for DDFS, $P = .59$ for OS). When a threshold at insulin ≤ 50 pmol/L versus > 50 pmol/L was modeled, hazard ratios for high versus low levels were 2.3 (95% CI, 1.5 to 3.7) for distant recurrence and 3.4 (95% CI, 1.9 to 6.2) for death. Modeling bending points at insulin levels of 40 and 60 pmol/L (ie, allowing an increase in risk between these values, with plateaus before and after) resulted in hazard ratios for insulin > 60 versus < 40 pmol/L of 2.6 (95% CI, 1.5 to 4.3) for distant recurrence and 3.8 (95% CI, 2.0 to 7.3) for death. Regardless of the threshold or bending points used, all models identified significant prognostic associations of insulin. Furthermore, the linear model yielded conservative estimates of hazard ratios over the range considered. Although it is possible a nonlinear relationship (eg, a threshold effect) exists for the association of insulin with recurrence and death, we cannot confirm this with existing data, and we have used the more conservative linear models in all analyses.

The nature of the relationship between BMI and breast cancer outcomes was also examined. When the log hazard was modeled linearly, BMI predicted DDFS ($P = .047$) and marginally predicted OS ($P = .063$) (data not shown). A J-shaped relationship has been previously reported as best reflecting this association.[36] We modeled this relationship by using a quadratic term in our Cox model. This approach represented a significant improvement over the linear model ($P < .001$), and it identified a significant relationship for both outcomes ($P < .001$ for DDFS and OS); women with either low or high BMI (ie, < 20 or > 25 kg/m^2) had the worst outcomes (Fig 2). Because we were confirming a previously reported relationship between BMI and outcomes and because this approach described our data most effectively, the quadratic term was used in all survival analyses.

Age was a significant predictor of DDFS but not OS ($P = .03$ and .23, respectively) in the entire study population. It

Table 3. Unadjusted Prognostic Effects at Quartile Midpoints

	Quartile Midpoint	Distant Recurrence		Death	
		Hazard Ratio	95% CI	Hazard Ratio	95% CI
Insulin, pmol/L, n = 504					
Q1, 8.1-27.0	21.4	1.0		1.0	
Q2, 27.0-35.3	31.1	1.3	1.1-1.5	1.5	1.2-1.8
Q3, 35.3-51.9	42.3	1.5	1.1-2.1	2.0	1.4-2.9
Q4, 51.9-339.8	69.5	2.0	1.2-3.3	3.1	1.7-5.7
P		.007		.001	
IGF-I,* ng/L, n = 512					
Q1, 26.4-119.0	98.9	1.0		1.0	
Q2, 119.0-151.4	137.0	1.17	0.98-1.40	1.02	0.80-1.29
Q3, 151.4-192.4	170.0	1.32	0.96-1.81	1.03	0.67-1.57
Q4, 192.4-391.5	221.7	1.55	0.93-2.59	1.04	0.53-2.05
P		.09		.90	
IGF-II,* ng/L, n = 503					
Q1, 267-516	473	1.0		1.0	
Q2, 516-583	554	1.00	0.83-1.20	0.98	0.77-1.24
Q3, 583-653	613	0.99	0.72-1.36	0.96	0.64-1.43
Q4, 653-954	703	0.99	0.60-1.63	0.94	0.50-1.77
P		.97		.84	
Estradiol,† pmol/L, n = 512					
Q1, 0-73.6	36	1.0		1.0	
Q2, 73.6-149	102	0.95	0.76-1.18	0.98	0.73-1.31
Q3, 149-301	224	0.92	0.62-1.35	0.97	0.58-1.61
Q4, 301-1257	498	0.88	0.51-1.53	0.95	0.46-1.98
P		.65		.9	
BMI, kg/m^2, n = 512					
Q1, 16.3-21.9	20.5	1.18	0.93-1.48	1.21	0.92-1.59
Q2, 21.9-24.5	23.1	1.0		1.0	
Q3, 24.5-27.8	26.1	1.10	0.97-1.26	1.10	0.94-1.29
Q4, 27.8-54.8	31.1	1.72	1.27-2.34	1.78	1.25-2.53
P		< .001		< .001	

NOTE. *P* values were obtained from Cox models; a quadratic polynomial was used for BMI. Quartile midpoints in the hazard ratio calculation are the 12.5th, 37.5th, 62.5th, and 87.5th percentiles of each variable. The second quartile of BMI was chosen as the reference category because it was associated with the lowest risk.

Abbreviation: Q, quartile.

*Prognostic effects remained nonsignificant after adjustment for IGFBP-3 levels in multivariate Cox models; IGF-I: $P = .48$ DDFS, $P = .83$ OS; IGF-I: $P = .21$ DDFS, $P = .84$ OS.

†No significant effect on DDFS or OS in postmenopausal subgroup ($P = .46$ and .21, respectively).

was significantly associated with both DDFS and OS in premenopausal ($P = .03$ and .01, respectively) but not in postmenopausal women ($P = .48$ and .38, respectively). Menopausal status was not significantly associated with either DDFS or OS ($P = .20$ and .62, respectively), in keeping with other reports.[37]

The prognostic importance of most traditional prognostic factors was confirmed. For tumor stage, the hazard ratio for T2 compared with T1 tumors was 2.5 (95% CI, 1.6 to 4.1) for DDFS and 3.2 (95% CI, 1.7 to 6.1) for OS; for T3 compared with T1 tumors, these hazard ratios were 3.4 (95% CI, 1.6 to 7.1) and 3.2 (95% CI, 1.1 to 8.8), respectively. For nodal stage, the hazard ratio for N1 compared with N0 was 3.0 (95% CI, 1.9 to 4.8) and 2.7 (95% CI, 1.5 to 4.8) for DDFS and OS, respectively. For tumor grade, the hazard ratio for grade 3 compared with grade 1 or 2 tumors was 1.9 (95% CI, 1.2 to 3.0) and 1.9 (95% CI, 1.0 to 3.5) for DDFS and OS, respectively. Hazard ratios for nuclear grade 3 compared with grade 1 or 2 were 1.7 (95% CI, 1.0 to 2.9) and 1.8 (95% CI, 0.9 to 3.5) for DDFS and OS, respectively. The presence of lymphatic invasion was associated with hazard ratios of 2.6 (95% CI, 1.5 to 4.4) and 2.2 (95% CI, 1.1 to 4.4) for DDFS and OS, respectively. Hazard ratios for ER-positive tumors or equivocal versus ER-negative tumors were 1.5 (95% CI, 0.9 to 2.6) and 3.1 (95% CI, 1.7 to 5.7) for DDFS and OS, respectively; whereas those for PgR-positive tumors or equivocal versus PgR-negative tumors were 2.1 (95% CI, 1.3 to 3.3) and 3.2 (95% CI, 1.7 to 5.9) for DDFS and OS, respectively. Hazard ratios were similar for at least one hormone receptor–positive tumors or equivocal versus both hormone receptors–negative tumors (1.5 [95% CI, 0.9 to 2.7] and 2.7 [95% CI, 1.4 to 5.2] for DDFS and OS, respectively).

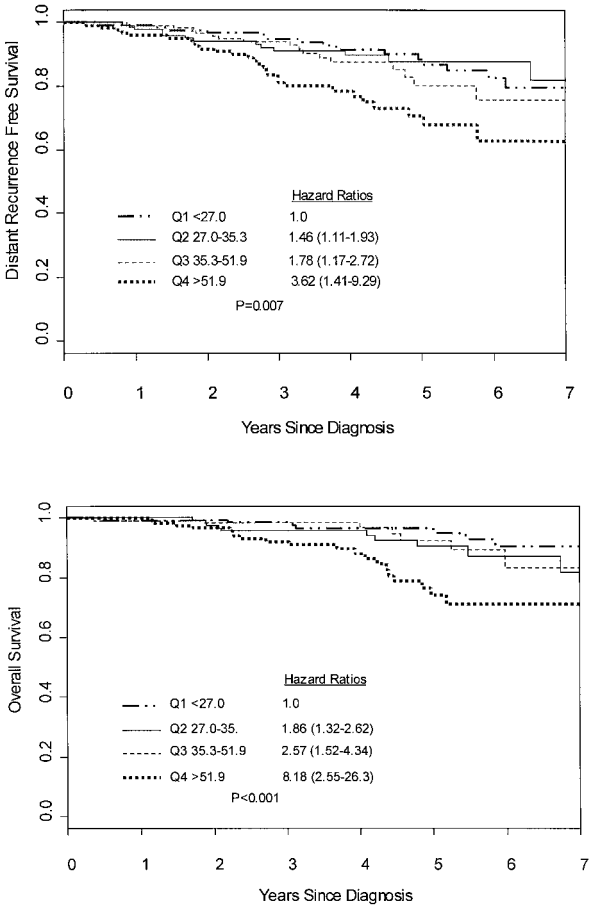

Fig 1. Kaplan-Meier estimates of distant recurrence-free survival (upper panel) and OS (lower panel) according to quartiles (Q) of fasting insulin (ng/L). Hazard ratio and *P* values are taken from Cox models of DDFS and OS, in which insulin was treated as a continuous variable.

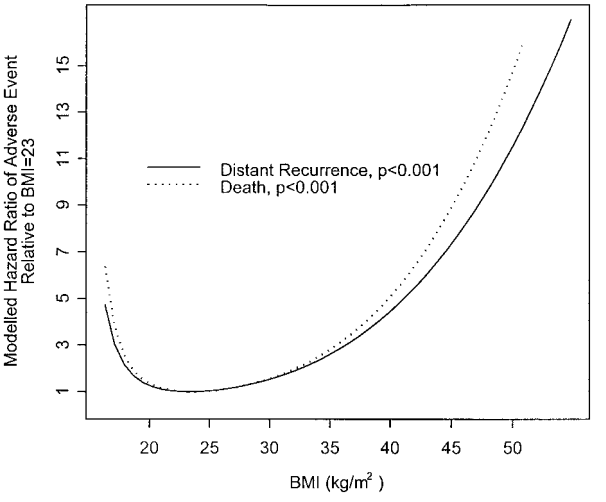

Fig 2. Hazard ratio functions for distant recurrence and death as a function of BMI, when the log hazard is modelled as a quadratic polynomial function of BMI in Cox models. *P* values are for the whole quadratic model.

Prognostic Associations of Insulin After Adjustment for BMI

Insulin and BMI were strongly correlated ($r = 0.59$). As a result, we did not expect their effects on breast cancer outcomes would be independent of each other. Nonetheless, in a Cox model that included both variables, insulin was significantly associated with OS after adjustment for BMI (hazard ratio upper versus lower quartile 3.2; 95% CI, 1.4 to 7.1). The effect of insulin on DDFS was modestly reduced after adjustment for BMI (hazard ratio upper versus lower quartile 1.7; 95% CI, 0.9 to 3.1). To illustrate this in a descriptive fashion, prognostic effects of insulin within different categories of BMI were examined. The hazard ratio of distant recurrence and death in upper versus lower quartiles of insulin were 1.9 and 3.1 in overweight women ($BMI > 25$ kg/m^2), 2.4 and 6.1 in normal weight women (20 kg/m$^2 \leq BMI \leq 25$ kg/m^2), and 1.4 and 2.4 in underweight women ($BMI < 20$ kg/m^2), respectively. It can be seen that, in each category, higher levels of insulin were associated with poorer outcomes, consistent with the existence of a prognostic effect of insulin across broad categories of body weight.

Exploration of the Effect of Tumor and Treatment Variables on the Prognostic Associations of Insulin

As shown above, insulin levels were significantly related to most traditional prognostic factors. To investigate whether prognostic associations of insulin persisted after consideration of these factors, we undertook a series of exploratory analyses. The design of these analyses was guided by the extent of missing data and, in the case of multivariate modeling, by the need to balance the number of variables included in a single model with the number of events observed (76 distant recurrences and 45 deaths in the entire population). Information on lymphatic invasion and nuclear grade was missing in 28.3% and 29.7% of women, respectively; furthermore, these data were available in a nonrepresentative subgroup of women, being significantly more likely to be missing in women with good prognosis tumors not receiving adjuvant chemotherapy. As a result, these two variables were not included in these exploratory analyses.

We began by examining prognostic associations of insulin after adjustment for each of these variables in turn. This allowed us to maximize the number of subjects included in each analysis. The hazard ratio for distant recurrence and death rose with increasing quartiles of insulin after adjustment for each variable. Furthermore, insulin was associated with DDFS and OS (hazard ratio for upper *v* lower quartile) after adjust-

Table 4. Cox Multivariate Analysis of DDFS and OS (n = 362)

	DDFS			OS		
	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Insulin, Q4 *v* Q1	2.1	1.2-3.6	.01	3.3	1.5-7.0	.002
Age, 35 *v* 50 years	1.5	0.9-2.5	.10	1.5	0.8-2.9	.20
Nodal stage, N1 *v* N0	3.4	1.8-6.6	< .001	2.3	1.0-5.3	.05
Tumor stage,* T3 *v* T1	1.4	0.6-1.9	.78	1.9	0.6-6.3	.52
Tumor grade,* 3 *v* 1	5.9	1.4-25.7	.01	2.5	0.5-11.7	.44
Hormone receptor,† negative *v* positive	1.4	0.7-2.6	.35	2.8	1.3-6.0	.01
Adjuvant chemotherapy, yes *v* no	0.5	0.2-1.0	.04	0.7	0.3-1.8	.51
Adjuvant tamoxifen, yes *v* no	0.6	0.3-1.1	.10	1.0	0.4-2.3	.93

Abbreviation: Q, quartile.

*Represented by two dummy variables in the model.

†Positive, either ER- or PgR-positive or equivocal, negative, both ER- and PgR-negative. When these receptors were entered as separate variables, PgR was significantly associated with both DDFS and OS (ER was not), and insulin remained significantly associated with both DDFS and OS. The combined variable was used to reduce the total number of variables in the models.

ment for tumor stage (1.8; 95% CI, 1.1 to 2.9 for DDFS and 2.7; 95% CI, 1.4 to 5.1 for OS), tumor grade (1.9; 95% CI, 1.1 to 3.2 for DDFS and 3.3; 95% CI, 1.7 to 6.6 for OS), nodal stage (1.7; 95% CI, 1.1 to 2.9 for DDFS and 2.8; 95% CI, 1.5 to 5.1 for OS) or ER (1.7; 95% CI, 1.0 to 2.9 for DDFS and 2.5; 95% CI, 1.3 to 5.0 for OS), and PgR (1.6; 95% CI, 0.9 to 2.8 for DDFS and 2.5; 95% CI, 1.3 to 5.0 for OS). Thus, there was little evidence in these exploratory analyses that prognostic associations of insulin were due solely to insulin's associations with individual traditional prognostic factors.

Additionally, insulin remained associated with prognosis after adjustment for adjuvant chemotherapy (either anthracycline-based or nonanthracycline-based), high levels of insulin being significantly associated with distant recurrence (hazard ratio for upper *v* lower quartile = 1.9; 95% CI, 1.2 to 3.2) and death (hazard ratio for upper *v* lower quartile = 3.0; 95% CI, 1.6 to 5.7). Insulin also remained prognostically important after adjustment for the effects of adjuvant tamoxifen (hazard ratio for upper *v* lower quartile of insulin = 2.1; 95% CI, 1.3 to 3.4 for DDFS and 3.1; 95% CI, 1.7 to 5.9 for OS). Simultaneous adjustment for adjuvant chemotherapy and/or tamoxifen yielded similar results. There was no evidence of interaction between insulin levels and adjuvant therapy (chemotherapy, tamoxifen, and both, all $P > .20$) in these prognostic analyses.

Exploratory Cox multivariate analyses were then performed including insulin, the most well accepted of these traditional prognostic factors that were available in our data set (age, axillary nodal stage, tumor stage, tumor grade, and hormone receptor status) and adjuvant systemic treatment (chemotherapy and hormone therapy). Our purpose was to identify, in an exploratory fashion, those factors most strongly associated with DDFS and OS. Because we could not include other potentially important factors (eg, nuclear grade and lymphatic invasion, as well as new molecular markers that were not measured in our study), we view our analysis as hypothesis-generating rather than a definitive examination of the contribution of insulin beyond that of all known prognostic factors. The results of these analyses are listed in Table 4. It can be seen that insulin remained significantly associated with both DDFS and OS in these models, adjusted hazard ratios being 2.1 (95% CI, 1.2 to 3.6) and 3.3 (95% CI, 1.5 to 7.0), respectively, very similar to those seen in unadjusted analyses.

DISCUSSION

We have identified an adverse prognostic association of high levels of fasting insulin measured shortly after surgical treatment of early-stage breast cancer (T1 to T3, N0 to N1, and M0) in women without known diabetes. Women with insulin levels in the uppermost quartile had a two-fold increased risk of distant recurrence and a three-fold increased risk of death compared with those with levels in the lowermost quartile. There was some evidence that insulin might be associated with breast cancer outcomes in a nonlinear fashion, for example, that a threshold effect might be present at insulin levels around 50 pmol/L or that there may be plateaus of risk above or below certain levels of insulin. We were not able to confirm this in our study; however, we did see an increased risk of recurrence and death with higher insulin levels regardless of the nature of the relationship we modeled. Future research should explore this further.

We performed a series of adjusted analyses to explore the effects of confounding by other variables that could have led to the identification of spurious prognostic effects of insulin. Insulin remained significantly associated with outcomes after adjustment for age and most traditional prognostic factors, including tumor and nodal stage, tumor

grade, and hormone receptor status. Thus, the association of insulin with outcome does not seem to be due solely to insulin's relationship with one (or more) of these traditional prognostic variables, although we were not able to fully explore effects of nuclear grade and lymphatic invasion. The association of insulin with distant recurrence and death also persisted after adjustment for adjuvant chemotherapy and hormone therapy. As in all observational studies, other variables we have not measured (eg, *HER2/neu* and *p53*) or unknown confounders may exist that are responsible for the prognostic associations of insulin we have identified. Furthermore, although significant interactions between insulin and other prognostic variables were not seen in our study, power may have been insufficient to detect some important interactions. As a result, our multivariate analyses should be considered exploratory; further research in independent data sets is needed to confirm our observations. For similar reasons, our survival analyses should be repeated after longer follow-up when more events (particularly deaths) have occurred, given the relatively small number of events included in this analysis (76 recurrences and 45 deaths).

Our study was performed in healthy women without known diabetes; we did not measure fasting glucose in these women. As a result, it is possible some of our study subjects had undiagnosed diabetes. Because this likely occurred in only a small number of women (if any) and because we excluded known diabetics, our results should not be extrapolated to women with diabetes, particularly those who use exogenous insulin who may have extreme insulin levels. Breast cancers in diabetic women develop in a physiologic environment very different from that of our subjects, and these cancers may have different characteristics from the cancers we have studied.

We have confirmed the association of BMI with breast cancer outcomes, a J-shaped relationship being present, in keeping with previous reports in breast cancer[36] and the general population.[38] BMI was strongly associated with insulin. We had postulated that high insulin levels resulted, to a large extent, from obesity, and that insulin mediated the prognostic effect of BMI in breast cancer. As a result, adjustment of the prognostic effect of insulin for BMI was expected to ameliorate insulin's effect and to represent potential overadjustment. Nonetheless, the contributions of insulin and BMI to OS were independently significant.

High insulin levels were most common in overweight women but were also seen in some normal-weight women. One potential mechanism for higher levels of insulin in overweight women is obesity-associated insulin resistance. It is possible that insulin resistance as a result of other factors is present in normal-weight women. Although we suspect that high insulin levels in our subjects reflect underlying insulin resistance, we have not been able to investigate whether insulin resistance was present in our study subjects. This should be a priority for future research.

We were not able to address the issue of whether more aggressive treatment might overcome the prognostic association of insulin we have observed. Women with higher insulin levels, who seem to be at increased risk of recurrence and death despite standard adjuvant therapies, should be the focus of trials that investigate the efficacy of more aggressive treatments using standard antineoplastic agents. Such trials should measure, and stratify or adjust for, fasting insulin levels in survival analyses, and they should examine the effect of the treatments used on insulin levels.

There is a strong biologic rationale for an adverse prognostic effect of insulin.[5,6] Up to 90% of breast cancer cells express IGF-I,[39-42] IGF-II,[43] insulin,[5-7] and/or hybrid insulin/IGF-I[44] receptors. IGF-I and insulin receptors, both of which have been reported to mediate the mitogenic effects of insulin,[5,6] are frequently overexpressed in breast cancer, with levels of insulin receptor (IR) that are six- to 10-fold higher than in normal breast epithelium having been reported. This overexpression may confer a selective growth advantage to breast cancer cells, especially in the presence of hyperinsulinemia, such as occurs in obesity or other insulin-resistant states. Furthermore, the IR-A isoform of the insulin receptor (differentiated from the B-isoform by the absence of a 12–amino acid segment at the carboxy terminal of the alpha subunit) has been reported to be the predominant insulin receptor in some breast cancers.[45] The enhanced sensitivity of this isoform to the mitogenic effects of insulin may contribute to potent mitogenic effects of insulin in breast cancer and to the strong adverse prognostic effects reported here. We have not been able to examine the role of insulin and IGF-I receptors as mediators of these prognostic effects nor have we been able to investigate signalling pathways for these mitogenic effects. One recent report suggests that insulin receptor substrate-1 is the predominant signalling molecule for both insulin and IGF-I in human breast cancer,[46] and there is evidence to suggest that there is cross-talk between IGF and estrogen-signaling pathways.[47] Based on current knowledge, we cannot conclude that the prognostic association of insulin we have identified is because of a direct effect of insulin on breast cancer cells. Further research to examine insulin-receptor interactions and subsequent signal transduction in breast cancer cells will be needed.

New therapeutic approaches that lower insulin levels, interfere with receptor-ligand interactions, or disrupt signalling pathways should be explored in the future, particularly if additional research supports a causal effect of insulin on breast cancer outcomes. Investigation of insulin physiology in women with breast cancer is likely to confirm the presence of insulin resistance, potentially leading to the development of strategies such as weight loss, regular

physical activity, or pharmacologic approaches that increase insulin sensitivity in normal tissues. The effect on breast cancer outcomes of reduction in insulin levels could then be examined. Alternatively, the development of specific receptor blockers that target the A isoform of the IR may be beneficial in cancers in which this isoform predominates. Research to understand signalling pathways responsible for the mitogenic effects of insulin in breast cancer cells may also lead to the development of novel therapeutic approaches. This research will enhance understanding of the nature of the prognostic effect of insulin, and it will contribute to the development of new therapeutic approaches.

IGF-I, a factor closely related to insulin, has received considerable attention in breast cancer.[9,48-50] Elevated levels have been associated with an increased risk of breast cancer, and IGF-I receptors are present on most breast cancer cells.[39-42] Commonly used treatments for breast cancer, such as tamoxifen, influence IGF-I;[51,52] however, circulating IGF-I has not been associated with a worsened prognosis[53] or consistently with adverse prognostic factors.[54,55] Overexpression of IGF-I[56] and its receptor[41,57,58] in breast cancer seems to be associated with improved outcomes, possibly reflecting up-regulation of receptors or better differentiation. Our failure to identify a significant adverse prognostic effect of IGF-I (or of IGF-II) may represent the true absence of a prognostic effect; alternatively, autocrine or paracrine production (not measured in our study) may lead to important prognostic effects that we have not investigated.

In summary, we have identified a previously unappreciated prognostic association in breast cancer. Although a cause-effect relationship between insulin and breast cancer outcomes cannot be proven in a single observational study, we believe the strength of the prognostic association we have identified, the persistence of this association after adjustment for potential confounders (including treatment), and the presence of a strong biologic rationale are consistent with a causal relationship. Additional research outlined above, including an examination of the prognostic effect of strategies that reduce insulin levels, will help to clarify whether the relationship of insulin to breast cancer outcomes is causal. In the meantime, our findings provide important prognostic information that should be explored in research settings. Measurement of insulin levels in clinical settings is not recommended until additional research confirms our findings and effective treatment strategies have been developed.

REFERENCES

1. Menon RK, Sperling MA: Insulin as a growth factor. Endocrinol Metab 25:633-647, 1996
2. Le Roith D: Insulin-like growth factors. N Engl J Med 336:633-640, 1997
3. Scheen AJ, Lefebvre PJ: Insulin action in man. Diabetes Metab 22:105-110, 1996
4. Whitelaw DC, Gilbey SG: Insulin resistance. Ann Clin Biochem 35:567-583, 1998
5. Belfiore A, Frittitta L, Costantino A, et al: Insulin receptors in breast cancer. Ann NY Acad Sci 784:173-188, 1996
6. Papa V, Belfiore A: Insulin receptors in breast cancer: Biological and clinical role. J Endocrinol Invest 19:324-333, 1996
7. Mathieu M-C, Clark GM, Allred DC, et al: Insulin receptor expression and clinical outcome in node-negative breast cancer. Proc Assoc Am Phys 109:565-571, 1997
8. Del Giudice ME, Fantus IG, Ezzat S, et al: Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat 47:111-120, 1998
9. Bruning PF, Bonfrer JMG, van Noord PAH, et al: Insulin resistance and breast-cancer risk. Int J Cancer 52:511-516, 1992
10. Goodwin PJ, Boyd NF: Body size and breast cancer prognosis: A critical review. Breast Cancer Res Treat 16:205-214, 1990
11. Tretli S, Haldorsen T, Ottestad L: The effect of pre-morbid height and weight on the survival of breast cancer patients. Br J Cancer 62:299-303, 1990
12. Camoriano JK, Loprinzi CL, Ingle JN, et al: Weight change in women treated with adjuvant therapy or observed following mastectomy for node-positive breast cancer. J Clin Oncol 8:1327-1334, 1990
13. Kyogoku S, Hirohata T, Takeshita Y, et al: Survival of breast-cancer patients and body size indicators. Int J Cancer 46:824-831, 1990
14. Kimura M: Obesity as prognostic factors in breast cancer. Diabetes Res Clin Pract 10:S247-S251, 1990
15. Coates RJ, Clark WS, Eley JW, et al: Race, nutritional status, and survival from breast cancer. J Natl Cancer Inst 82:1684-1692, 1990
16. Ewertz M, Gillanders S, Meyer L, et al: Survival of breast cancer patients in relation to factors which affect the risk of developing breast cancer. Int J Cancer 49:526-530, 1991
17. Nomura AMY, LeMarchand L, Kolonel LN, et al: The effect of dietary fat on breast cancer survival among Caucasian and Japanese women in Hawaii. Breast Cancer Res Treat 18:S135-S141, 1991
18. Vatten LJ, Foss OP, Kvinnsland S: Overall survival of breast cancer patients in relation to preclinically determined total serum cholesterol, body mass index, height and cigarette smoking: A population-based study. Eur J Cancer 27:641-646, 1991
19. Senie RT, Rosen PP, Rhodes P, et al: Obesity at diagnosis of breast carcinoma influences duration of disease-free survival. Ann Int Med 116:26-32, 1992
20. Gordon NH, Crowe JP, Brumberg DJ, et al: Socioeconomic factors and race in breast cancer recurrence and survival. Am J Epidemiol 135:609-618, 1992
21. Tornberg S, Carstensen J: Serum beta-lipoprotein, serum cholesterol and Quetelet's index as predictors for survival of breast cancer patients. Eur J Cancer 29A:2025-2030, 1993
22. Bastarrachea J, Hortobagyi GN, Smith TL, et al: Obesity as an adverse prognostic factor for patients receiving adjuvant chemotherapy for breast cancer. Ann Intern Med 119:18-25, 1993
23. Katoh A, Watzlaf VJM, D'Amico F: An examination of obesity and breast cancer survival in post-menopausal women. Br J Cancer 70:928-933, 1994

24. Obermair A, Kurz C, Hanzal E, et al: The influence of obesity on the disease-free survival in primary breast cancer. Anticancer Res 15:2265-2270, 1995

25. Den Tonkelaar I, de Waard F, Seidell JC, et al: Obesity and subcutaneous fat patterning in relation to survival of postmenopausal breast cancer patients participating in the DOM-project. Breast Cancer Res Treat 34:129-137, 1995

26. Zhang S, Folsom AR, Sellers TA, et al: Better breast cancer survival for postmenopausal women who are less overweight and eat less fat. Cancer 76:275-283, 1995

27. Maehle BO, Tretli S: Pre-morbid body-mass-index in breast cancer: Reversed effect on survival in hormone receptor negative patients. Breast Cancer Res Treat 41:123-130, 1996

28. Rosner GL, Hargis JB, Hollis DR, et al: Relationship between toxicity and obesity in women receiving adjuvant chemotherapy for breast cancer: Results from Cancer and Leukemia Group B study 8541. J Clin Oncol 14:3000-3008, 1996

29. Haybittle J, Houghton J, Baum M: Social class and weight as prognostic factors in early breast cancer. Br J Cancer 75:729-733, 1997

30. Block G, Hartman A, Dressor CN, et al: A data based approach to diet questionnaire design and testing. Am J Epidemiol 124:453-459, 1986

31. Levine MN, Pritchard KI, Bramwell V, et al: A randomized trial of cyclophosphamide, epirubicin, fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, fluorouracil in premenopausal women with node-positive breast cancer. J Clin Oncol 16:2651-2658, 1998

32. Fisher B, Redmond C, Legault-Poisson S, et al: Postoperative chemotherapy and tamoxifen alone in the treatment of positive node breast cancer patients 50 years and older with tumours responsive to tamoxifen: Results from National Surgical Breast and Bowel Project B-16. J Clin Oncol 8:1005-1018, 1990

33. Bonadonna G, Brusamolino E, Valagussa P, et al: Combination chemotherapy as adjuvant treatment in operable breast cancer. N Engl J Med 294:405-410, 1976

34. McNair DM, Lorr M, Droppleman LF: EdITS Manual for the Profile of Mood States, Revised 1992. San Diego, CA, EdITS/ Educational and Industrial Testing Service, 1992

35. Horowitz M, Wilner N, Alvarez W: Impact of Event Scale: A measure of subjective stress. Psychosomatic Med 41:209-218, 1979

36. Suissa S, Pollak M, Spitzer WO, et al: Body size and breast cancer prognosis: A statistical explanation of the discrepancies. Cancer Res 49:3113-3116, 1989

37. Jatoi I, Hilsenbeck SG, Clark GM, et al: Significance of axillary lymph node metastasis in primary breast cancer. J Clin Oncol 17:2334-2340, 1999

38. Calle EE, Thun MJ, Petrelli JM, et al: Body-mass index and mortality in a prospective cohort study of U.S. adults. N Engl J Med 341:1097-1105, 1999

39. Railo MJ, Smitten KV, Pekonen F: The prognostic value of insulin-like growth factor-I in breast cancer patients: Results of a follow-up study on 126 patients. Eur J Cancer 30A:307-311, 1994

40. Foekens JA, Portengen H, van Putten WLJ, et al: Prognostic values of receptors for insulin-like growth factor 1, somatostatin and epidermal growth factor in human breast cancer. Cancer Res 49:7002-7009, 1989

41. Happerfield LC, Miles DW, Barnes DM, et al: The localization of the insulin-like growth factor receptor 1 (IGFR-1) in benign and malignant breast tissue. J Pathol 183:412-417, 1997

42. Papa V, Gliozzo B, Clark GM, et al: Insulin-like growth factor-I receptors are overexpressed and predict a low risk in human breast cancer. Cancer Res 53:3736-3740, 1993

43. Sleat DE, Chen T-L, Raska K, et al: Increased levels of glycoproteins containing mannose 6-phosphate in human breast carcinomas. Cancer Res 55:3424-3430, 1995

44. Pandini G, Vigneri R, Costantino A, et al: Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: Evidence for a second mechanism of IGF-I signaling. Clin Cancer Res 5:1935-1944, 1999

45. Frasca F, Pandini G, Scalia P, et al: Insulin receptor isoform A, a newly recognized high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 19:3278-3288, 1999

46. Zhang X, Yee D: Tyrosine kinase signalling in breast cancer: Insulin-like growth factors and their receptors in breast cancer. Breast Cancer Res 2:170-175, 2000

47. Jackson JG, White MF, Yee D: Insulin receptor substrate-1 is the predominant signalling molecule activated by insulin-like growth factor-1, insulin and interleukin-4 in estrogen receptor-positive human breast cancer cells. J Biol Chem 273:9994-10003, 1998

48. Hankinson SE, Willett WC, Colditz GA, et al: Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351:1393-1396, 1998

49. Peyrat JP, Bonneterre J, Hecquet B, et al: Plasma insulin-like growth-factor-I (IGF-I) concentrations in human breast cancer. Eur J Cancer 29A:492-497, 1993

50. Yee D: The insulin-like growth factors and breast cancer revisited. Br Cancer Res Treat 47:197-199, 1998

51. Canobbio L, Cannata D, Miglietta L, et al: Somatuline (BIM 23014) and tamoxifen treatment of postmenopausal breast cancer patients: Clinical activity and effect on insulin-like growth factor-I (IGF-I) levels. Anticancer Res 15:2687-2690, 1995

52. Ho G-H, Ji C-Y, Phang B-H, et al: Tamoxifen alters levels of serum insulin-like growth factors and binding proteins in postmenopausal breast cancer patients: A prospective paired cohort study. Ann Surg Onc 5:361-367, 1998

53. Bhatavdekar JM, Patel DD, Karelia NH, et al: Tumour makers in patients with advanced breast cancer as prognosticators: A preliminary study. Br Cancer Res Treat 30:293-297, 1994

54. Barni S, Lissoni P, Brivio F, et al: Serum levels of insulin-like growth factor-I in operable breast cancer in relation to the main prognostic variables and their perioperative changes in relation to those of prolactin. Tumouri 80:212-215, 1994

55. Vadgama JV, Wu Y, Datta G, et al: Plasma insulin-like growth factor-I and serum IGF-binding protein 3 can be associated with the progression of breast cancer, and predict the risk of recurrence and the probability of survival in African-American and Hispanic women. Oncol 57:330-340, 1999

56. Toropainen E, Lipponen P, Syrjanen K: Expression of insulin-like growth factor I (IGF-I) in female breast cancer as related to established prognostic factors and long-term prognosis. Eur J Cancer 31A:1443-1448, 1995

57. Bonneterre J, Peyat JP, Beuscart R, et al: Prognostic significance of insulin-like growth factor 1 receptors in human breast cancer. Cancer Res 50:6931-6935, 1990

58. Peyrat JP, Bonneterre PH, Vennin H, et al: Insulin-like growth factor I receptors (IGF1-R) and IGF1 in human breast tumours. J Steroid Biochem Mol Biol 37:823-827, 1990