Effect of Raloxifene on Insulin-Like Growth Factor-I, Insulin-Like Growth Factor Binding Protein-3, and Leptin in Premenopausal Women at High Risk for Developing Breast Cancer

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Abstract

Elevated insulin-like growth factor I (IGF-I) is associated with an increased risk for developing breast cancer in premenopausal women, whereas lower leptin levels have been documented in premenopausal breast cancer cases. We determined the effect of raloxifene on IGF-I, insulin-like growth factor binding protein 3 (IGFBP-3), and leptin in premenopausal women at high risk for developing invasive breast cancer.

Twenty-eight premenopausal women (median age 43 years) participating in a pilot breast cancer prevention trial provided 56 matched serum samples. Specimens were collected at baseline and after treatment with 60 mg of raloxifene daily. Median treatment duration was 3 months (range: 6 weeks to 12 months). Samples were frozen at $-70°C$ until analysis. IGF-I, IGFBP-3, and leptin were measured by ELISA. Significance was evaluated by the Wilcoxon signed rank test.

Raloxifene administration increased serum IGFBP-3 [mean change 245 ng/ml; $P = 0.017$; 95% confidence interval (CI), 76–415] and leptin (mean change 2.1 ng/ml; $P = 0.005$; 95% CI, 0.6–3.7). No significant change in serum IGF-I was detected (mean change 2.6 ng/ml; $P = 0.84$; 95% CI, -15.4 to 20.6). IGF-I:IGFBP-3 molar ratio was stable (mean change -0.014; $P = 0.30$; 95% CI, -0.041 to 0.012).

Raloxifene administration is associated with an increase in IGFBP-3 and leptin in premenopausal high risk women. Increases in IGFBP-3 may potentially decrease the activity of circulating IGF-I. The effect of modulating the IGF pathway and leptin on breast cancer risk needs additional evaluation.

Introduction

Insulin-like growth factor (IGF) -I and leptin are hormones vital to normal development and yet, similar to estrogen, they are also implicated in breast carcinogenesis. In normal physiology these proteins are integral to sexual development, energy balance, and nutrition. IGF-I is necessary for breast development and skeletal growth (1). The IGF binding proteins (IGFBPs) modulate the bioavailability of IGF-I (2), and IGFBP-3 is its primary carrying protein (3). Similarly, leptin levels correlate with hormonal events in women: pubertal development, (4) pregnancy, and the menstrual cycle (5). *In vitro*, leptin stimulates the growth of human breast epithelial cells (6). Moreover, whereas circulating leptin levels reflect fat stores and body mass index, estrogen causes release of leptin from adipocytes, and women have higher levels of leptin, even when controlled for percentage of body fat, than men (7). Thus, IGF-I, IGFBP-3, and leptin are integral to multiple hormonally modulated events that may contribute to breast cancer risk.

The complex interactions among the IGF pathway, leptin, and estrogens are also implicated in the pathological process of breast cancer development. A number of investigators have demonstrated that estrogen receptor-positive breast cancer cell lines proliferate in response to treatment with IGF-I and leptin, whereas estrogen receptor-negative cells do not grow in the presence of IGF-I, and their response to leptin is unknown (6, 8–10). In breast tumor models, IGF-I and estrogen have been found to have a reciprocal relationship (11, 12). IGF-I and ER appear to promote the expression of each other, although the mechanism is not clearly defined. Other components of the IGF pathway may also contribute to breast carcinogenesis. In addition to binding IGF-I, IGFBP-3 has independent actions important in tumor growth. IGFBP-3 can mediate apoptosis in breast cancer cell lines, (13, 14) and inhibits estrogen-mediated growth of breast cancer cells (15).

Epidemiology studies provide corroborative evidence for the hypothesis that IGF-I and leptin are growth factors for breast cancer. Two prospective case-control studies have demonstrated that elevated levels of circulating IGF-I are a risk factor for breast cancer in premenopausal women and, in addition, one of the groups of investigators found that IGFBP-3 had an inverse relationship with cancer incidence, although this was not statistically significant (16, 17). The data on leptin as a breast cancer risk factor is less robust. One small case control study found decreased leptin levels in premenopausal breast cancer cases ($n = 14$) compared with controls ($n = 15$; Ref. 18), and in a larger study of premenopausal women with ductal carcinoma *in situ*, leptin levels were lower in cases than controls, although the relationship was inverted when the analysis was adjusted for BMI and other confounders (19). Older women (mean age 64) with breast cancer had higher leptin levels than controls (mean age 43; Ref. 20). However, taken

together, these preclinical and epidemiological studies suggest that the IGF pathway and leptin may be appropriate targets in the premenopausal breast cancer prevention setting.

Additional evidence from breast cancer treatment studies illustrate that the IGF pathway and leptin can be targeted by hormonal agents. Tamoxifen, a selective estrogen receptor modulator (SERM) approved for breast cancer prevention, affects both the IGF pathway and leptin (21, 22). IGF-I levels decrease in women with breast cancer on tamoxifen treatment, whereas leptin levels increase (23). We evaluated the effect of raloxifene on IGF-I, IGFBP-3, and leptin levels in premenopausal women at increased risk for breast cancer participating in a pilot chemoprevention study. Raloxifene is currently indicated for the prevention and treatment of osteoporosis in postmenopausal women. There is inadequate safety information on the use of raloxifene in premenopausal women. Raloxifene was studied because of its association with reduced breast cancer incidence (24). The currently accruing Study of Tamoxifen and Raloxifene trial is assessing the effectiveness of tamoxifen *versus* raloxifene as a breast cancer prevention agent. However, this trial does not include premenopausal women (25). The effects of raloxifene, a selective estrogen receptor modulator, on IGF-I, IGFBP-3, and leptin levels is of interest, because these proteins may represent potential surrogate biomarkers of cancer risk.

Materials and Methods

Patient Population. Patient samples were collected from premenopausal women at high risk for invasive breast cancer enrolled on a pilot prevention trial. Protocol 98-C-0123, a Phase II trial of raloxifene in premenopausal women at high risk for developing invasive breast cancer, was approved by the National Cancer Institute Institutional Review Board, and all of the participants provided written informed consent. Goals of this pilot trial included determining the effects of raloxifene on bone mineral density, the ovaries, and serum hormone levels, in addition to evaluating the effect of raloxifene on serum IGF-I, IGFBP-3, and leptin in the premenopausal setting. Evaluation of the effects of raloxifene on bone mineral density, the ovaries, uterus, and serum hormone levels are ongoing, and will be reported separately. Patients were enrolled on the study from December 1998 to January 2002. Eligible patients had an increased risk of breast cancer by at least one of the following factors: Gail model risk assessment ≥1.7% over 5 years, a family history consistent with hereditary breast cancer (26), a diagnosis of lobular carcinoma *in situ*, atypical ductal hyperplasia, or locally treated ductal carcinoma *in situ*. Subjects were required to have regular menstrual cycles (defined as 26–35 days) for the 6 months preceding enrollment in the trial. Premenopausal status was additionally verified by a follicle stimulating hormone level <20 mIU/ml. After enrolling on the trial and before starting raloxifene, menstrual regularity was again confirmed during a 1–2-month run-in period during which a menstrual cycle length of 26–35 days was verified by patient report, and ovulation was verified by luteal phase progesterone levels (2–20 ng/ml). Women who were not ovulatory during the run-in period were taken off study. Exclusion criteria included the use of any other hormonal medications including birth control pills, hormone replacement therapy, tamoxifen, progesterones, or corticosteroids.

Lifestyle Assessment. Exercise, tobacco use, and alcohol intake were assessed during the baseline evaluation and at the time of the on-treatment blood draw. Exercise was categorized as meeting the 1990/95 Centers for Disease Control recommen-dations for physical activity or not (27, 28). These categories include both moderate intensity exercise at more frequent intervals (6–7 days/week for at least 30 min) and higher intensity activity less often (3 days/week for 20–60 min). Tobacco use was categorized as either currently smoking or not. Alcohol intake was reported as glasses of wine, beer, or spirits typically consumed in 1 week. These estimates were then converted to grams/day (0.57 × the number of cocktails/week +0.44 × the number of beers/week +0.40 × the number of glasses of wine per week) × 28.35/7 (29). Nondrinkers reported no alcohol intake. All of the variables were assessed during an in-person interview with a member of the study team.

Treatment Plan. Subjects on study were prescribed raloxifene for 2 years. After stopping raloxifene subjects were followed off-drug for 1 year and underwent repeat follow up examinations. Subjects self-administered raloxifene 60 mg daily and calcium carbonate 1250 mg/day. Raloxifene was supplied by Eli Lilly and Co. Calcium was given to prevent age-related bone loss as per NIH Consensus Conference guidelines (30). Calcium was obtained from commercial sources.

Sample Collection. Fasting serum samples were collected at baseline, *i.e.,* before women started raloxifene, and again after being on raloxifene and calcium for ~3 months. When possible, samples were collected during the early follicular phase of the menstrual period (3–10 days after the start of menses). Serum was immediately separated and stored frozen at −70°C.

Assay Methods. Samples were analyzed for total IGF-I, IGFBP-3, and leptin by sandwich-type ELISAs (Diagnostic Systems Laboratories, Inc., Webster, TX). For IGF-I, duplicate aliquots of each serum sample were pretreated according to the manufacturer's instructions and assayed in duplicate; IGFBP-3 and leptin were also assayed in duplicate. Individual samples with coefficient of variation (%CV) >15% were reassayed as above, and values with %CV <15% were obtained. Statistical testing was done with both the original and repeated set of values. No statistically significant difference in results was seen between the two analyses (data not shown). Reported results are for tests with %CV <15%. The mean value of the results was used in the analysis.

Statistical Methods. Statistical significance between paired baseline and on raloxifene hormone levels was evaluated by the Wilcoxon signed rank test ($P = 0.05$). Correlations among IGF-I, IGFBP-3, and leptin, and age, BMI, Gail model risk, length of time on drug, or day of blood draw within the menstrual cycle were evaluated by Spearman rank correlation method. Linear regression and the Wald test of the regression coefficient were used in controlling for BMI in the leptin analysis.

Results

Thirty-seven women enrolled in the trial (see Fig. 1). During the run-in phase, 2 subjects withdrew: 1 for an intercurrent medical issue that interfered with baseline evaluations and 1 refused additional evaluations after signing consent. Additionally 1 did not have IGF-I, IGFBP-3, and leptin levels drawn at baseline but continued on study. Thirty-four subjects had baseline serum collected, and of these 5 did not start raloxifene: 1 for logistical reasons, 1 decided on a surgical prevention intervention, 1 for an intercurrent medical issue, 1 for anovulation during the run-in period, and 1 for work-up of a suspicious breast nodule. Of the 29 remaining subjects, 1 stopped raloxifene after 22 days. The reported findings are for the 28 patients who were able to provide matched samples. These 28 did not
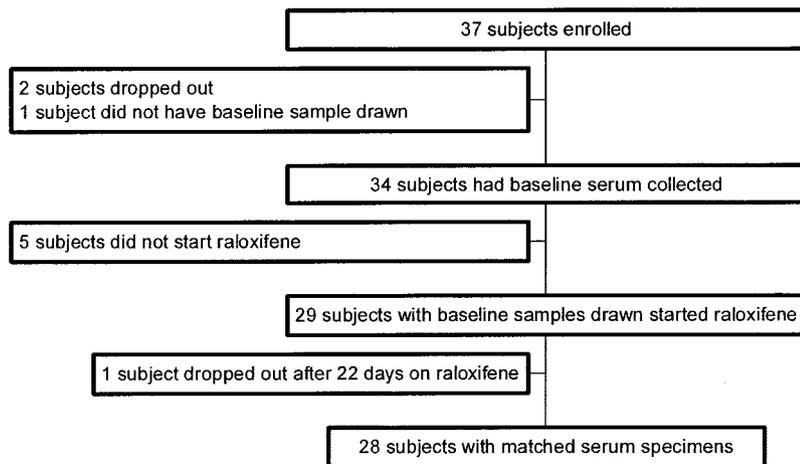
Fig. 1. Participant flow diagram.

significantly differ in baseline IGF-I and IGFBP-3 from the entire cohort of 34 (data not shown). Characteristics of these 28 women are shown in Table 1. Median time of taking raloxifene was 3 months (range, 6 weeks to 12 months). Of samples, 96% (54 of 56) were collected during the early follicular phase of the menstrual period. Lifestyle characteristics that may affect IGF-I, IGFBP-3, and leptin are shown in Table 2. There was minimal change in these factors from the baseline evaluation to the time that the on-raloxifene sample was collected.

Baseline IGF-I ranged from 80 ng/ml to 317 ng/ml. For IGF-I assays, the %CV was 4.6% ±5.3 SD. No significant change in serum IGF-I on raloxifene was detected (mean change 2.6 ng/ml; $P = 0.84$; 95% CI, −15.4 to 20.6; see Table 3). Baseline IGFBP-3 ranged in value from 1858 ng/ml to 4125 ng/ml. The %CV for IGFBP-3 assays was 6.2 ±4.2 SD. Raloxifene administration was associated with a statistically significant increase in IGFBP-3 (mean change 245 ng/ml; $P = 0.017$; 95% CI, 76−415). IGF-I:IGFBP-3 molar ratio was stable (mean change −0.014; $P = 0.30$; 95% CI, −0.041 to 0.012). Additionally, no correlations were found among IGF-I, IGFBP-3, and age at baseline, BMI, interval between blood draws, time on drug, day of menstrual cycle of blood draw, or Gail model risk.

Baseline leptin levels ranged from 2.5 ng/ml to 49.8 ng/ml and correlated with BMI, as expected. The %CV for leptin assays was 4.4 ±3.2 SD. Leptin levels increased significantly while on raloxifene (mean change, 2.1 ng/ml; $P = 0.005$; 95% CI, 0.6−3.7). Nineteen of the changes were positive, 8 were negative, and 1 was zero. The change in leptin level had no correlation with the baseline leptin level ($P > 0.80$). Of note, the 1 subject tested after 6 weeks on raloxifene had no change in her level. The subject tested at 1 year had a decrease in her leptin of 4.2 ng/ml; however, she also lost 12 kg over that time period. When we adjusted for BMI using a number of linear regression models, the magnitude of the change of leptin while on raloxifene decreased; however, the increase in leptin with raloxifene was still statistically significant ($P < 0.03$ by the Wald test).

Discussion

Elevated IGF-I has been associated with an increased risk of developing breast cancer in premenopausal women (16, 17). Additionally, decreased leptin levels have been associated with an increased risk for invasive breast cancer in premenopausal women (18). We found that raloxifene, a SERM and promising breast cancer prevention agent, caused a statistically significant increase in IGFBP-3 and leptin in premenopausal women at high risk for breast cancer. No significant effect on IGF-I or IGF-I:IGFBP-3 molar ratios was observed.

Our findings are novel, because no other published trials have evaluated the effect of SERMs on IGF-I, IGFBP-3, and leptin simultaneously in a premenopausal at-risk population. However, these agents have been evaluated in the postmenopausal setting. In postmenopausal breast cancer patients, tamoxifen and raloxifene each decrease serum IGF-I. A significant decrease in IGF-I was seen in postmenopausal women with metastatic breast cancer on tamoxifen (21) and in a neoadjuvant trial using raloxifene; no effect on IGFBP-3 was seen (31). Similarly, healthy women who had undergone hysterectomy experienced a decrease in IGF-I and no significant change in IGFBP-3 when treated with tamoxifen (22).

We observed a different effect on the IGF pathway in the premenopausal setting than that seen in the postmenopausal situation. Rather than lowering IGF-I, raloxifene increased IGFBP-3 and had no statistically significant effect on the IGF-I:IGFBP-3 molar ratio in our population. However, the IGF-I:IGFBP-3 molar ratio slightly decreased, and the lack of significant effect may be due to the small sample size. The finding that raloxifene increased IGFBP-3, whereas having no effect on

Table 1	Baseline characteristics of study population
Total	28
Median age (years)	43 (range, 35–47)
Race/ethnicity	
Caucasian	27
Hispanic	1
BMI (kg/m²)[a,b]	24.6 (range, 19.2–40.8)
High risk by	
Gail model	22 (avg risk, 2.6)
DCIS	3
LCIS	3
Family history	1
FSH (mIU/ml)	6.3
Luteal phase progesterone (ng/ml)	12.5

[a] BMI, body mass index; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; FSH, follicle stimulating hormone.
[b] BMI, FSH, and luteal phase progesterone are average values.
[c] Subject may have been eligible by more than one high-risk criteria.

Table 2 Lifestyle characteristics of study population

	Baseline	On raloxifene
Exercise		
Yes[a]	22	21
No[b]	6	7
Tobacco use		
Current user	3	3
Nonuser	25	25
Alcohol intake (g/day)		
Nondrinker	11	11
<5	14	14
5.0–15	2	2
>15	1	1

[a] Meets 1990/95 Centers for Disease Control (CDC) recommendations for physical activity.
[b] Does not meet 1990/95 CDC recommendations for physical activity.

IGF-I may be in keeping with the well-recognized agonist/antagonist effects of this class of drugs, which can be dependent on menopausal status. For example, tamoxifen has agonist activity on the bones, increasing bone mineral density in postmenopausal women but antagonist activity on the bones in the premenopausal setting (32). Another class of potential chemopreventive agents, the retinoids, also exhibits differential effects on the IGF pathway by menopausal status. In a randomized study of early breast cancer patients, use of a synthetic vitamin A analogue, fenretinide, was associated with a significant increase in IGFBP-3 in all women, whereas changes in IGF-I were dependent on menopausal status (33). In premenopausal women a decline in IGF-I was noted; however, in postmenopausal women there was an upward trend in IGF-I levels. Thus, it appears that the IGF system can be targeted by different types of agents, which have different effects based on menopausal status, implicating the importance of the hormonal environment. Although the chemical or molecular structure of an individual SERM may determine its effects on the IGF system, it still remains that the same general trends were seen with both tamoxifen and raloxifene, and that menopausal status was the most notable difference in the trials to date.

Preclinical data have shown similar effects of hormonal agents on IGFBP-3, and these findings support the concept that changing IGFBP-3 levels may influence breast cancer growth. *In vitro* and animal studies have shown that SERMs and antiestrogens can increase IGFBP-3 alone. In MCF-7 cells, treatment with either tamoxifen or ICI 182,780 (fulvestrant) inhibits cell growth and increases IGFBP-3, suggesting that this binding protein may have antiproliferative actions (34). In a healthy ovary intact rat model, fulvestrant increases IGFBP-3 mRNA in a dose-dependent fashion, whereas tamoxifen does so to a lesser degree (34, 35). The effect of the antiestrogens is opposite that of estrogen itself, which decreases IGFBP-3 production (36, 37). This additionally highlights the complex cross-talk between estrogen and the IGF pathway. In addition to estrogen and IGF-I working in concert in normal and pathological states, estrogen can moderate the IGF pathway further by interacting with other members of the IGF family.

In regard to leptin in breast cancer treatment studies, tamoxifen increases levels of leptin in breast cancer patients compared with those who were not taking tamoxifen. This group included both premenopausal and postmenopausal women with breast cancer (38). Another treatment trial in postmenopausal women only demonstrated that leptin levels increased after raloxifene treatment was initiated (23). Although the effect of a SERM on leptin has not been demonstrated previously in an exclusively premenopausal high risk group of women, our results are comparable with effects seen on leptin in the treatment setting, *i.e.,* raloxifene, like tamoxifen, increased circulating leptin. The effect of SERMs on leptin appears to be the same in pre- and postmenopausal women in these early studies. Whether this increase in leptin will result in a decreased incidence of breast cancer is a hypothesis that needs additional testing.

Other factors affect IGF-I, IGFBP-3, and leptin levels. One of the primary modulators is BMI. However, there was no significant change in BMI in our study participants to explain the increased leptin levels and when BMI was controlled for, the increase in leptin levels on raloxifene was still statistically significant. Other confounders include amount of exercise, smoking, and alcohol use, and none of these appreciably changed during the time between the two sample collections. Another potential confounder is the use of calcium in our cohort. A recent study suggested that foods high in calcium and vitamin D were associated with a reduced risk of breast cancer in premenopausal women (39), and there is emerging evidence that foods in this category, *e.g.,* skim milk, can increase IGF-I, IGFBP-3, and the IGF-I:IGFBP-3 molar ratio (40). Although there is currently no definitive evidence that calcium supplements alone would result in increased IGFBP-3 levels, we cannot exclude this possibility. The effect of calcium supplements on leptin is unknown. Other genomic factors have been linked with IGFBP-3 protein expression. A single nucleotide polymorphism in the promoter region of IGFBP-3 at the -202 locus has been correlated with circulating IGFBP-3 levels (41). Moreover, this genotype interacts with alcohol intake (negatively) and retinol levels (positively) to affect IGFBP-3 levels (41, 42). Thus, the increase in IGFBP-3 seen in our study may be mediated through this type of mechanism. However, it seems unlikely that our small cohort with heterogeneous risk profiles would have commonality in an IGFBP-3 single nucleotide polymorphism that would lead to a statistically significant change in IGFBP-3 levels, although we cannot definitively exclude this possibility. Additionally, there may be an unrecognized confounder that could lead to spurious results, yet on the whole our study was well controlled for known confounders.

Table 3 Effect of raloxifene on serum IGF-I,[a] IGFBP-3, and leptin levels

Serum levels	Baseline[b]	On raloxifene[b]	Mean change[c]	P	95% CI
IGF-I ng/ml	189	192	2.6	0.84	−15.4, 20.6
IGFBP-3 ng/ml	2785	2991	245	0.017	76, 415
IGF-I:IGFBP-3 molar ratio	0.26	0.25	−0.014	0.30	−0.041, 0.012
Leptin ng/ml	15.6	17.8	2.1	0.005	0.6, 3.7

[a] IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; CI, confidence interval.
[b] Baseline and On raloxifene are mean values.
[c] Mean change is the average of intrasubject change.

Our findings are important, because we are evaluating potential intermediate biomarkers of breast cancer. A key factor in doing this successfully is having a well-defined population. We were able to achieve this through stringent inclusion criteria and specimen collection protocols. As indicated above, the IGF pathway and leptin appear to correlate with sex hormones, and reproductive events. Thus, to additionally understand the link between the hormonal milieu and these energy balance markers, we will determine whether changes seen in IGFBP-3 and leptin correlate with changes in sex hormone levels using samples obtained over the course of the menstrual cycle. Another fundamental issue in determining the utility of surrogate end points is being able to evaluate their effect at the target organ, in this case breast tissue. In the future we will evaluate tissue effects in our cohort.

Our results provide evidence that modulating the IGF pathway is achievable with a potential breast cancer prevention agent. No other studies to date have prospectively evaluated the effects of a SERM on the IGF pathway and leptin in a carefully defined premenopausal high-risk population. If the IGF pathway can be modulated by potential chemopreventive agents, this may prove to be a useful surrogate end point biomarker and provide insight about the pathophysiology of breast cancer. Moreover, because these hormones are intricately tied to pathways that can be influenced by lifestyle changes, *e.g.,* weight loss, their role in disease development warrants special interest because they may be modifiable by nonpharmaceutical measures. In the prevention setting, IGF-I, IGFBP-3, and leptin may be part of a more extensive serum panel that helps identify an at-risk woman and predict what intervention may be of benefit. While in the treatment realm, obesity may predict poor prognosis for both pre- and postmenopausal women (43–45), and understanding if leptin exerts a negative effect in the continuum of breast cancer progression, which could be modulated, would be important. Additional research is needed to better understand the mechanisms of these proteins, particularly leptin, and their action in breast tissue.

Acknowledgments

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Effect of Raloxifene on Insulin-Like Growth Factor-I, Insulin-Like Growth Factor Binding Protein-3, and Leptin in Premenopausal Women at High Risk for Developing Breast Cancer

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