SHORT COMMUNICATION

Baseline Leptin Levels Predict Change in Leptin Levels During Weight Loss in Obese Breast Cancer Survivors

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■ **Abstract:** Leptin is an adipocyte-derived hormone involved in regulation of satiety, and it also appears to have a role in breast cancer risk. Leptin therefore might be a useful indicator of the potential preventive effects of weight loss in breast cancer survivors. In this study we examined whether the change in leptin levels could be predicted by weight loss in obese breast cancer survivors. The subjects in this study were participating in a randomized trial of an individualized approach towards weight loss in Detroit, MI. Breast cancer survivors (body mass index of 30–44 kg/m^2) were enrolled and fasting blood samples were obtained for leptin analysis over 1 year of study. Leptin levels were available from at least two time points for 36 women, and weight change ranged from a gain of 11% to a loss of 25% of baseline weight. Using a repeated-measures regression model, both baseline leptin level and concurrent percent body fat were found to synergistically predict leptin levels. Thus, for women with the same body fat, those with higher baseline leptin levels are predicted to exhibit smaller decreases in leptin with weight loss. Similar results were obtained for body weight and body weight change, but the associations with body fat were stronger. Breast cancer survivors with initially higher leptin levels may differ with regard to regulation of change in leptin during weight loss resulting in relatively smaller changes in leptin with equivalent amounts of weight loss. ■

Key Words: breast cancer, leptin, obesity, weight loss

Weight gain leading to overweight and obesity is becoming a world-wide epidemic that has increased dramatically in prevalence during recent years (1). This is a matter of great importance for the treatment of breast cancer as recurrence rates and survival in early-stage disease are both negatively impacted by increased body weight in the vast majority of studies that have been conducted (2–4). Unlike the effects of obesity on de novo breast cancer risk, which is different in pre- and postmenopausal women, the adverse effect of increased body weight on recurrence risk and survival has been evident in both pre- and postmenopausal women (3,5). For example, in a large study of young women, higher body mass was associated with tumors of higher cellular proliferation and decreased survival from breast cancer (6). Obesity also has been shown to increase the risk of contralateral breast cancer (7).

Weight gain after treatment for breast cancer can exacerbate pre-existing overweight. Women diagnosed with breast cancer have been observed to gain weight, which can negatively affect health status in survivorship (8–11). In a Canadian study, weight gain after a diagnosis of breast cancer was greater in women treated with chemotherapy (2.5 kg in the year after diagnosis) than with surgery and/or hormonal therapy alone (0.6 kg) (12). One small study showed that weight gain in breast cancer patients was not greater than that in control women; however, the breast cancer patients were unique in that there was a significant increase in body fat 6 months after chemotherapy (13). Other studies also have shown that chemotherapy for breast cancer results in sarcopenic weight gain, perhaps as a result of decreased physical activity levels (10,14,15).

Several metabolic alterations associated with the adverse health effects of increased body weight, and in particular an increase in body fat, may affect breast cancer recurrence risk. These include the effects of obesity on insulin levels and oxidative stress (16–18). Elevated leptin levels also have been associated with increased breast cancer risk and poor prognosis in breast cancer (19,20), although not all studies agree (21–24). Leptin is, however, associated with insulin resistance, breast cancer cell proliferation, and possibly anti-estrogen resistance (25–28). Leptin is an obesity-related hormone secreted by adipose tissue, and it is decreased following weight loss. The physiological role of leptin may include signaling an increase in fat stores

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to suppress hunger (29). In obese persons, however, leptin resistance develops. Leptin resistance has been suggested to occur at levels higher than 25 ng/mL as transport of leptin to the brain is saturated at those levels (29). Thus, very high levels of leptin might not be incrementally effective for curbing energy intake. This brings into concern the observations made in most studies that breast cancer patients have significantly higher blood leptin levels than women without cancer, but this might not be the case in premenopausal women which is consistent with observations that obesity protects against premenopausal breast cancer risk (20,30–35). We reported previously that the obese breast cancer survivors in our study had higher leptin levels than those reported for breast cancer patients of mixed body weight status (36). In breast cancer patients, regulation of leptin pathways has not been investigated in as much detail as it has in other obese persons.

A number of mechanisms by which leptin can increase breast cancer risk are currently being examined. In vitro, leptin exerts proliferative effects in breast cancer cells (37–39). Leptin also appears to be angiogenic (19). In human breast tissues, leptin was detected in the cancerous tissue, the surrounding normal tissue, and in areas of atypical hyperplasia, but it was not localized in the normal breast (37). Leptin has therefore been hypothesized to be a reasonable target for interventions that aim to decrease breast cancer risk (40). Leptin decreased more in obese breast cancer survivors who lost 10% of their initial body weight versus those losing less weight over 12 months (36). A decrease in leptin levels, if disclosed to study subjects, might be a motivating factor to lose weight because of its relationship to breast cancer risk. Previous studies in obese women, however, have indicated that high leptin levels are associated with low resting energy expenditure thereby affecting the extent of weight loss achieved (41–43). It was therefore important to determine whether or not change in leptin levels can simply be predicted by extent of weight or body fat loss in obese breast cancer survivors or whether baseline leptin and body fat do affect the subsequent rate of change in leptin levels over time.

METHODS

Subjects

Eligible subjects were aged 18–70. Study subjects had stage I or II breast cancer diagnosed within the past 4 years and were free of any recurrence as confirmed by a physician. Chemotherapy or radiation therapy was to have been completed at least 3 months previously with the exception of tamoxifen. Recruitment sources included direct mail to Race for the Cure participants, press releases and brochures at breast clinics. The study was reviewed and approved by the Institutional Review Board of Wayne State University. Subjects gave informed, written consent to participate. Details of the study have been published previously (44), and in the present analysis 36 women who completed 12 months of study were included.

Forty-eight subjects were randomly assigned to one of four groups: control, Weight Watchers (WW), individualized counseling, or a combination of WW and individualized counseling. Nine of the forty-eight women did not complete 12 months of participation. Subjects in the control arm received the National Cancer Institute's "Action Guide to Healthy Eating" and the "Food Guide Pyramid" pamphlets, but they received no other dietary or exercise instructions or help. For the WW arm, women were given free coupons and encouraged to attend WW meetings but received no other dietary or exercise instruction. For the Individualized arm, women received telephone counseling with a registered dietitian. Finally, for the Combination arm, subjects received both individualized counseling and were asked to attend weekly WW meetings using free coupons. This resulted in a population of women with varying amounts of weight loss at 12 months.

Assessments

Women were asked to come to the appointments after an overnight fast and to drink two glasses of water in the morning. Plasma was prepared immediately after drawing blood at baseline, 3, 6 and 12 months and stored frozen at −70°C until analysis. Leptin levels were determined with commercial enzyme immunoassays kits using a high-affinity polyclonal capture antibody (Linco Research, St. Charles, MO). Levels of insulin were also measured by radioimmunoassay (ICN Biomedicals Inc., Costa Mesa, CA), and glucose levels were measured using a spectrophotometric assay with glucose oxidase (Sigma Chemical Co., St. Louis, MO). Questionnaires were administered at baseline and intervals thereafter to capture demographic and health factors. At baseline, 3, 6, 9 and 12 months, the women were weighed in clothing but without shoes using a Health-o-Meter

Professional Beam Scale, model 402KLS (Bridgeview, IL). Percent body fat was measured using tetrapolar bioelectrical impedance (model BIA101S; RJL Systems, Clinton Township, MI). Height was measured at baseline only.

Statistical Methods

The data were analyzed using SAS 9.1 software (SAS Inc., Cary, NC). The data were normally distributed and no transformation was needed prior to conducting the analyses. Descriptive analyses were performed using *t*-tests and other appropriate exploratory measures. For the main statistical analyses, we used repeated-measures regression models with either leptin or rate of change in leptin as the outcome measure. The single within-subject factor used in the model was time. Both body fat (or rate of change in body fat) and baseline leptin levels were used as covariates in the model. Tukey–Cramer adjustment was used for post-hoc pairwise comparison between the time-points. An unstructured within-subject correlation matrix was used for the purpose of flexibility. These models were also constructed using body weight and weight change instead of body fat and body fat change.

RESULTS AND DISCUSSION

Subjects and Baseline Leptin Levels

There were 36 subjects on whom at least two leptin levels were available. Of the 48 women enrolled, six dropped out before a second blood sample could be obtained and another six did not have more than one blood sample available due to difficulties with the phlebotomy that are common in this population. For the 36 subjects in this analysis, the mean age was 52 years (SD 9, range 36–69), BMI at baseline was 35 kg/m^2 (SD 4, range 30–44), and only four of the women were pre-menopausal. The majority of the 36 women were white; five were African-American and one was native American. Of the 36 women, 11 gained weight over 12 months of the study and the rest lost weight. Weight loss was a mean of 4.9% of baseline weight at 12 months (SD 8.6%, range a gain of 11.3% to a loss of 27.2%). Body fat was measured by tetrapolar bioelectric impedance. This method may underestimate body fat in obese persons relative to dual energy x-ray absorptiometry, but it is sensitive enough to detect changes in body fat (45). At baseline, mean body fat was 39.4% (SD 2.9, $n = 36$, range 47–34) and after 12 months it was 37.5% (SD 3.8, $n = 36$, range 44–25).

At baseline, leptin levels were available for 35 women as blood was not available from one woman at baseline. The mean levels were 40.3 ng/mL (SD 17.5) for 24 women taking tamoxifen and 33.6 ng/mL (SD 15.5) for 11 women not taking tamoxifen ($p = 0.286$ from *t*-test for differences between the two groups), and 38 ng/mL (SD 17) for all 35 women combined. Other studies have also found higher levels of leptin in breast cancer patients taking tamoxifen versus those not taking tamoxifen (34,46). After 12 months on study, mean leptin was 34 ng/mL (SD 19, $n = 31$). As a reference, American females in the National Health and Nutrition Examination Survey (NHANES III) had mean leptin levels of 26 and 35.4 ng/mL for women of BMI 30–35 and over 35 kg/m^2, respectively, and mean leptin levels of 9.9 ng/mL for women in the normal weight range (47).

Change in Leptin Levels

The pattern of change in leptin levels generally followed the change in body fat and body weight. Leptin levels in women classified by weight change category are shown in Fig. 1. Leptin decreased in most of the 13 women who lost weight without any regain. A loss followed by gain (regain in weight ranged 0.9–9 pounds) occurred in 16 women during 12 months, and in these women leptin levels generally mirrored the change in weight. Seven women steadily increased weight from baseline to 12 months. Leptin increased in four of these seven women and leptin decreased in the remaining three women, two of whom subsequently displayed a weight loss at 18 months. Note that it is possible that some women may have gained and lost weight in between visits, or vice versa, which in turn could have affected observed leptin levels. The data overall agree with published studies indicating that leptin decreases with decreases in body weight (48).

We then modeled unadjusted leptin levels as a function of the concurrent body fat values. This was a 3-point repeated-measures regression model, with leptin values at 3, 6, and 12 months as the outcome. Time was used as a within-subject factor in the model. The covariates used in the model were the concurrent body fat measurements at 3, 6, and 12 months, and the baseline leptin measure for each subject. As indicated in Table 1, concurrent body fat percentage ($p = 0.002$) and baseline leptin level ($p = 0.044$) were both positively associated with subsequent leptin levels.

There was also a significant difference found across measurements at different time points ($p = 0.006$). As is shown in Table 1 under ''time effects'', from month 3 onwards leptin levels followed an increasing trend. A post hoc pairwise comparison with Tukey–Cramer adjustment revealed that leptin levels at month 3 were significantly lower than that at month 6 ($p = 0.008$). The other time changes were not significant ($p > 0.1$). This indicates that there was a prominent drop in mean leptin level between 0 and 3 months followed by an overall increase in the later months. Controlling the regression model either individually or collectively for baseline tamoxifen use, insulin resistance (as fasting glucose/insulin ratio), and waist-hip ratio did not make any qualitative change in the findings.

We further explored how (if at all) change in body fat would predict change in the leptin levels. We formed the rate of change in leptin by subtracting the baseline leptin measure from the 3, 6, and 12 month measures and divided the raw change values by the month of observation. This gave us a ''rate of change'' in leptin per month, and adjusted for unequal observation periods in comparing the change-scores. Using this as the outcome variable, and baseline leptin level, concurrent body fat change, and time (as a categorical variable with three levels) as independent variables, the analysis was carried out under a repeated-measures regression framework. The changes in body fat values were constructed in a manner similar to the leptin changes. Both baseline leptin level as well as body fat change predicted change in leptin levels. The results are shown in Table 1. The positive estimate corresponding to the body fat variable indicated that the change in leptin and change in body fat followed a similar pattern ($p < 0.0001$). On the other hand, the negative estimate corresponding to the baseline leptin level signified that a higher starting level of leptin was associated with a lower overall change in leptin values ($p = 0.004$). Thus, for persons with the same body fat, those individuals with higher baseline leptin would be predicted to decrease leptin levels to a smaller extent.

The overall effect of the within-subject factor time was also found to be significant ($p = 0.004$). As shown in Table 1, the negative regression coefficients corresponding to the time effects indicated that rate of

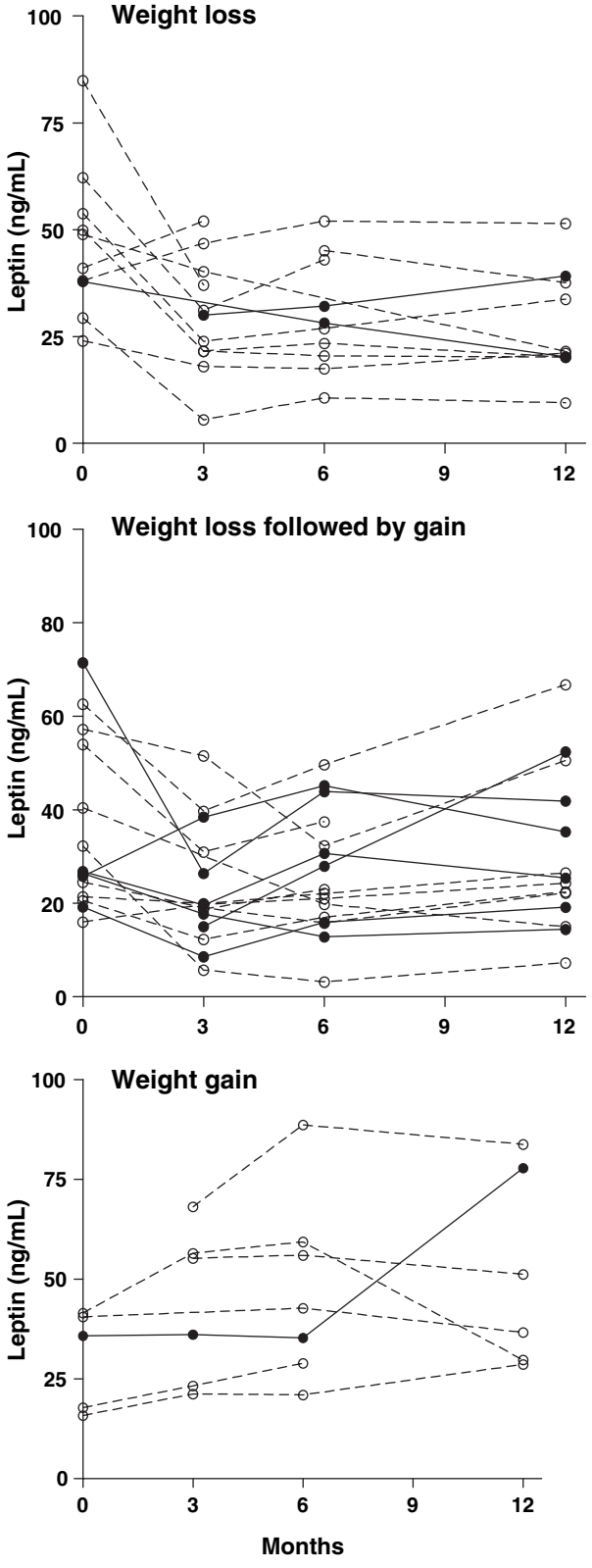

Figure 1. Change in body weight and plasma leptin levels with time in breast cancer survivors who were taking tamoxifen (open circles, dashed line) or not taking tamoxifen (solid circles, solid line). Graphs shown are for subjects who lost weight without regain, lost and regained weight or gained weight over 12 months of the study.

Table 1. Regression Coefficients (β), Standard Errors (SE), and Corresponding p-values for Repeated-Measures Regression Models with either (1) Leptin Levels or (2) Rate of Change in Leptin Levels as the Outcomes

	Leptin levels*†			Change in leptin levels†‡		
Independent variables	β	SE	p-value	β	SE	p-value
Overall effects						
Percent body fat	1.701	0.525	0.002	—	—	—
Rate of change in body fat	—	—	—	2.463	0.549	<0.0001
Baseline leptin	0.269	0.128	0.044	−0.037	0.012	0.004
Time effects						
Months 3–6	−4.988	1.517	0.008	−2.310	0.636	0.003
Months 3–12	−5.921	2.846	0.114	−2.577	0.844	0.013
Months 6–12	−0.934	2.879	0.944	−0.267	0.379	0.763

*Change in leptin levels with time.
†Covariates for the model with leptin level as the outcome were percent body fat, baseline leptin, and time (months). Covariates for the model with rate of change in leptin level as the outcome were rate of change in body fat, baseline leptin, and time (months).
‡Rate of change in leptin levels per month from baseline.

change in leptin levels slowed with time. Once again, the indication was that there was a sharp decrease in levels within the first 3 months into the trial followed by a flattening effect after 6 months. The pairwise comparison indicated both the 6 and 12 month rate of changes to be significantly slower than the 3 month rate of changes, while the 6 and 12 month rates not differing appreciably from each other. This is consistent with the slower rate of weight loss, and weight regain in some subjects, observed from 6 to 12 months. Once again, controlling for baseline tamoxifen use, insulin resistance, and waist-hip ratio as potential confounders did not change the findings.

Very similar results were obtained with positive and significant estimates for the overall effects of body weight ($b = 0.197$, $p = 0.001$) and body weight change ($b = 0.595$, $p < 0.0001$) on leptin levels. Again there was a negative estimate for baseline leptin (estimate -0.0363, $p = 0.0022$) indicating that higher baseline leptin levels were associated with less change in leptin over time in persons with the same body weight. The finding of smaller decreases in leptin in persons with initially higher leptin levels did not appear to be a result of a smaller weight loss in persons with the initially high leptin levels. Persons with higher leptin generally weighed more at baseline (Pearson correlation coefficient $r = 0.420$) and lost more weight (average weight loss at 12 months was 5.8% and 1.9% of baseline weight for initial leptin levels of >25 versus <25 ng/mL, respectively). When using body weight instead of body fat in the models, the overall effect of the within-subject factor time was found to be of only borderline significance. As leptin is secreted by adipocytes, it is not surprising that the relationships with body fat were stronger than with body weight.

There are several reasons why persons with relatively high levels of leptin initially might exhibit smaller decreases in leptin with weight loss. If indeed leptin resistance occurs above about 25 ng/mL (29), then regulation of leptin may differ in persons with high levels. In addition, leptin levels have been shown to be only partly explained by body fat (29). Limitations of this study are that the sample size was modest, and the number of variables examined therefore had to be limited. In addition, body fat was only measured by bioelectric impedance, which may underestimate body fat in obese persons (45). The results, however, do indicate that the effects of weight or body fat change on leptin levels are expected to differ by baseline leptin level. If leptin affects breast cancer risk, this brings into importance research into the factors that contribute to high leptin levels in breast cancer survivors.

CONCLUSIONS

Obese breast cancer survivors who had relatively high leptin levels for a given level of body fat at baseline exhibited smaller decreases in leptin after weight loss. Further investigation into the factors that contribute to high leptin levels is warranted given the possible importance of leptin in breast cancer outcomes.

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