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Associations of insulin-like growth factor and insulin-like growth factor binding protein-3 with mortality in women with breast cancer

Catherine Duggan, PhD[1], **Ching-Yun Wang, PhD**[1], **Marian L. Neuhouser, PhD**[1], **Liren Xiao, MSc**[1], **Ashley Wilder Smith, PhD, MPH**[2], **Kerryn W. Reding, PhD, MPH**[1,5], **Richard N. Baumgartner, PhD**[4], **Kathy B. Baumgartner, PhD**[4], **Leslie Bernstein, PhD**[3], **Rachel Ballard-Barbash, MD, MPH**[2], and **Anne McTiernan, MD, PhD**[1]

[1]Fred Hutchinson Cancer Research Center, Public Health Sciences, Seattle, WA [2]Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland [3]Division of Population Sciences, City of Hope National Medical Center, Duarte, CA [4]Department of Epidemiology and Population Health, School of Public Health and Information Sciences, University of Louisville, Louisville, KY [5]School of Nursing, University of Washington, Seattle, WA

Abstract

Elevated circulating insulin-like growth factor-1 (IGF-1), a breast epithelial cell mitogen, is associated with breast cancer development. However, its association with breast cancer survival is not established. Circulating concentrations of IGF-1 are controlled via binding proteins, including IGF Binding Protein-3 (IGFBP-3), that may modulate the association of IGF1 with breast-cancer outcomes.

We measured IGF-1 and IGFBP-3 concentrations in serum from 600 women enrolled in the Health, Eating, Activity, and Lifestyle (HEAL) Study, a multiethnic, prospective cohort study of women diagnosed with stage I-IIIA breast cancer. We evaluated the association between IGF-1 and IGFBP-3, and as a ratio, modeled using quintile cut-points, with risk of breast cancer-specific (n=42 deaths) and all-cause mortality (n=87 deaths) using Cox proportional hazards models. In models adjusted for body mass index, ethnicity, tamoxifen use at time of blood draw, treatment received at diagnosis, and IGFBP-3, women in the highest quintile of IGF-1 level had an increased risk of all-cause mortality (Hazard Ratio (HR)=3.10 95% CI 1.21-7.93, p=0.02), although no dose-response association was evident. The IGF-1/IGFBP-3 ratio, an indicator of free IGF-I levels, was significantly associated with increasing risk of all-cause mortality (HR=2.83 95% CI 1.25-6.36 P_{trend}=0.01, upper vs. lower quintile) in a fully adjusted model.

In conclusion, high serum levels of IGF-1 and the IGF-1/IGFBP-3 ratio were associated with increased risk of all-cause mortality in women with breast cancer. These results need to be confirmed in larger breast cancer survivor cohorts.

Corresponding Author: Catherine Duggan, Public Health Sciences, Fred Hutchinson Cancer Research Center (FHCRC), MD-B306, Seattle, WA, cduggan@fhcrc.org, Tel: 206 667 2323.

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Evidence from some, but not all epidemiologic studies demonstrates an association between circulating insulin-like growth factor-1 (IGF-1) levels and increased risk of breast cancer. However data on the association between circulating IGF-1 levels and prognosis in breast cancer survivors are limited. Here we describe the associations between levels of IGF-1 and IGF-1 binding protein-3 (IGFBP-3) and survival in a cohort of breast cancer survivors.

Keywords

IGF-1; IGFBP-3; breast cancer survival; mortality

Introduction

Insulin resistance is associated with increased risk of mortality in women with breast cancer.[1, 2] IGF-1 shares significant sequence homology with insulin;[3] is a regulator of cell growth and metastasis;[4] is a potent mitogen for both normal and transformed breast epithelial cells, [4] and displays mitogenic and anti-apoptotic effects.[3] IGF-1 also activates gene transcription programs strongly associated with poor breast cancer prognosis.[5] Bioactivity of IGF-1 is regulated via IGF Binding Proteins (IGFBPs), and approximately 90% of the IGF-I in the circulation is bound to IGFBP-3,[6, 7] together with an acid-labile subunit, to form a ternary inactive complex. In addition to its growth-inhibiting properties via competitively binding to IGF-I, IGFBP-3 has intrinsic and independent growth inhibiting effects, such as induction of apoptosis.[8, 9] The IGF-1/IGFBP-3 molar ratio has been proposed as a measure of free unbound IGF-1.[10] IGF-1 is also downregulated by tamoxifen.[11]

Evidence from some, but not all epidemiologic studies demonstrates an association between circulating IGF-I levels and increased risk of breast cancer.[12-21] However data on the association between circulating IGF-1 levels and prognosis in breast cancer survivors are limited. One small study (N=110) reported an association between lower levels of IGF-1 and improved survival.[22] Another found no association between IGF-1 and outcome but did find a significant association between elevated IGFBP-3 levels and recurrence.[23] We hypothesized that higher levels of IGF-I and lower levels IGFBP-3 will be associated with increased breast-cancer and all-cause mortality. We also hypothesized that higher molar ratios of IGF-I/IGFBP-3, an indicator of free IGF-1 levels, would also be associated with poor outcome. Here, we examined associations between fasting serum levels of IGF-1 and IGFBP-3 and breast-cancer specific and all-cause mortality in the Health Eating Activity and Lifestyle (HEAL) study, a cohort of breast cancer survivors, diagnosed with stage I-IIIa breast cancer. We also analyzed IGF-1, IGFBP-3, and the IGF-I/IGFBP-3 ratio, using quintile cut points to identify any potential threshold effect on the risk of breast-cancer specific and all-cause mortality.

Materials and methods

Study Setting, Participants, and Recruitment

The Health, Eating, Activity, and Lifestyle (HEAL) Study is a population-based, multicenter, multiethnic prospective cohort study that has enrolled 1,183 women diagnosed with breast cancer to evaluate whether diet, weight, physical activity, lifestyle, hormones or other exposures affect breast cancer prognosis. The aims, study design and recruitment procedures have been published previously.[24]

Briefly, women were recruited into the HEAL study through Surveillance, Epidemiology, and End Results (SEER) registries in New Mexico, Los Angeles County (CA), and western Washington. Baseline surveys were conducted on average 6 months after diagnosis (Fig. 1). In New Mexico, we recruited 615 women, 18 years or older, diagnosed with *in situ* to Stage IIIA breast cancer between July 1996 and March 1999, and living in Bernalillo, Sante Fe, Sandoval, Valencia, or Taos Counties. In Western Washington, we recruited 202 women, between the age 40-64 years, diagnosed with *in situ* to Stage IIIA breast cancer between September 1997 and September 1998, and living in King, Pierce, or Snohomish Counties. In

Los Angeles County, we recruited 366 Black women with stage 0 to IIIA primary breast cancer, who had participated in the Los Angeles portion of the Women's Contraceptive and Reproductive Experiences (CARE) Study. Los Angeles participants were U.S.-born, English-speaking women aged 35-64 years and diagnosed with breast cancer between May 1995 and May 1998. Recruitment was restricted in Western Washington and Los Angeles County to women aged 35-64 at diagnosis because of competing studies and design of the parent study. The study was performed with the approval of the Institutional Review Boards of participating centers, in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was obtained from each subject.

A total of 944 women completed in-person interviews at approximately three years post-diagnosis. Of these, 612 had a diagnosis of local or regional breast cancer, with complete data available on adiponectin, insulin and glucose levels.

A total of 944 women completed in-person interviews 24 months following their first interview (approximately 3-years post-diagnosis), at which time fasting blood samples were drawn.

Analysis was restricted to 628 women who had a diagnosis of local or regional breast cancer (we excluded 188 women with a diagnosis of Stage 0 (*in situ*) disease), with complete data available on IGF-1 and IGFBP-3 levels. An additional 28 women were excluded; 25 who had non-fatal breast cancer events <9 months before their 24-month interview dates to avoid potential confounding from possible recent treatment, and 3 with blood glucose levels <3.0 mmol/L (5.4 mg/dL) indicating possible incorrect specimen handling.[25, 26] The final sample is 600 participants; including 58 participants with a diagnosis of diabetes.

Data Collection and Covariates

Specimens

A 30-mL fasting blood sample was collected from patients at the 24-month interview, processed within 3 hours of collection, and stored at −80° C until analysis.

Assays

IGF-1 and IGFBP-3 measurement in HEAL participants, including intra-assay coefficient of variation at different concentrations, has been described in detail.[27] Briefly, IGF-1 and IGFBP-3 were assayed using radioimmunoassays from Nichols Institute Diagnostics (San Clemente, CA, USA) with a sensitivity of 0.1 ng/mL and 0.0625 μg/mL, respectively. IGF-1 and IGFBP-3 were analyzed at the USC Endocrine Research Laboratory for participants recruited in Los Angeles; and at the University of New Mexico for other participants. All samples were randomly assigned to assay batches and randomly ordered within each batch. Laboratory staff were blinded to subject identity. Serum insulin, glucose and Homeostatic Model Assessment (HOMA) scores, a measure of insulin resistance, were measured or calculated as described previously.[1]

Covariates

Standardized information, obtained during an interview, including medical history, demographic and lifestyle information, was collected at baseline (corresponding to 6 months post diagnosis), and at 24-months. Disease stage, estrogen (ER) receptor status and adjuvant therapy details were abstracted from medical records. At the 24-month interview, weight was measured to the nearest 0.1 kg with participants wearing light indoor clothing, and height was measured, without shoes, to the nearest 0.1 cm. Measurements were performed

twice, and averaged. Body mass index (BMI) was calculated as kg/m^2. A race/ethnicity/study-site 4-category variable was created as race/ethnicity and study-site were highly correlated: Non-Hispanic whites in New Mexico; non-Hispanic whites in Western Washington; Hispanics; African Americans.

Stage of Disease and Cancer Treatment

Participants were classified as having Stage 0 (*in situ*), Stage I (localized) or Stage II-IIIA (regional) breast cancer based on AJCC stage of disease classification contained within SEER. This analysis includes only women with Stage I-IIIa at diagnoses. Estrogen receptor (ER) status of tumors was categorized as positive, negative, or unknown/borderline. Treatment and additional clinical data were obtained from medical record reviews. Treatment was categorized into 3 groups: surgery only, surgery plus radiation, or surgery with any chemotherapy with or without radiation.

Outcome Assessment

Information on vital status was obtained from SEER. Cause of death codes were acquired from linkages with relevant SEER databases, which obtain data from state and national death certificates and the National Death Index. Studies examining the accuracy of death certificate data found that their use did not result in a meaningful change to mortality-based outcomes.[28] If alive, individuals were followed through their last follow-up assessment or SEER vital status update, whichever was most recent. All-cause mortality was defined as time from the 24-month follow-up interview (when serum samples were collected) to death from any cause, or end of follow-up (31 December 2007). Breast cancer-specific mortality was defined as death from breast cancer or end of follow-up, with the same intervals as for all-cause mortality.

Statistical Analysis

Differences in distribution of continuous variables between racial/ethnic groups, and other dichotomous patient characteristics were estimated using analysis of variance (ANOVA) with multiple comparison testing (Scheffé F-test). Differences in distributions of IGF-1 and IGFBP-3 between categorical variables were compared using the Chi-square test. Pearson correlation coefficients were obtained to represent associations between IGF-1 and IGFBP-3. IGF-1 and IGFBP-3 were not normally distributed, and were log-transformed. Both IGF-1 and IGFBP-3 were categorized using quintile cut-points. A ratio of IGF-1 to IGFBP-3 was also calculated, and categorized using quintile cut-points.

Hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer-specific or all-cause mortality were based on the partial likelihood for Cox's proportional hazards model.[29] The proportional hazard assumption was tested using Schoenfeld residuals, and no violation of the proportionality assumption was found. Age was used as the underlying time variable, with entry and exit time defined as the participant's age at the 24-month follow-up interview, and age at death from either breast cancer or any cause, or end of follow-up, respectively. We based variable inclusion on a likelihood ratio test, with the following covariates included in models: race/ethnicity/study-site; BMI (categorical <18.5 kg/m^2; ≥18.5 and <25 kg/m^2; ≥25 and <40 kg/m^2; ≥40 kg/m^2); tamoxifen use at time of blood draw (Yes/No); and treatment received at diagnosis (surgery; surgery+radiotherapy; chemotherapy). We included the race/ethnicity/study-site variable to adjust for different distributions of race/ethnicity by study site; this also adjusted for the fact that assays were carried out at two sites. Covariates considered but not included in the final model (as they did not significantly change the likelihood ratio score) were: menopausal status, education, smoking status, ER status and physical activity level.

We estimated the relationship between levels of IGF-1 and IGFBP-3 and breast cancer-specific mortality and all-cause mortality, using 2 different models: (1) adjusted for race/ethnicity/site, BMI, tamoxifen use at time of blood draw, and treatment; and (2) adjusted for model 1 covariates, with IGF-1 and IGFBP3 in the same model. As the literature suggests that the relationship between IGF-1, IGFBP-3, and IGF-1/IGFBP-3 ratio, and outcome may not be linear, we examined outcome by individual quintiles; comparisons for the upper 4 quintiles were made against the referent group (the lowest quintile).

We determined whether the association of IGF-1 and IGFBP-3 with outcomes were the same across subgroup categories, using a test of homogeneity of trends across groups; specifically stage, ER status; BMI≤25 and >25 (events were too few to investigate additional BMI subgroups); and tamoxifen use at time of blood draw. Due to sample size considerations, we generated a 2-level category, comparing levels of either IGF-1 or IGFBP-3 in the highest quintile, to quintiles 1-3 combined. Due to small numbers of events in premenopausal participants, it was not possible to compare pre- and postmenopausal subgroups.

All p-values are two-sided. Analyses were performed using STATA 11 (Statacorp, TX USA).

Results

Characteristics of HEAL participants are shown in Table 1. Median follow-up time was 94.7 months. Mean age at the 24 month follow-up interview was 57.6 years; mean BMI was 27.9 kg/m^2. Eighty-seven deaths occurred, of which 42 were due to breast-cancer. IGF-1 values were statistically significantly higher in Hispanics (P<0.0001) and Non-Hispanic whites (P<0.0001), than among African Americans. IGFBP-3 levels were significantly higher in non-Hispanic whites (P=0.003) than African Americans: differences between other racial/ethnic categories did not differ statistically. IGF-1 levels were significantly higher in participants not taking tamoxifen at time of blood draw (150.9 ng/mL standard deviation (s.d.) 68.3 ng/mL *vs.*111.7 ng/mL s.d. 47.7; (P=<0.0001), and in participants without a diagnosis of diabetes (132.7 ng/mL s.d. 61.2 *vs.* 110.7 ng/mL s.d. 63.2; P=0.01). Levels of IGF-1 were highest in women with a BMI ≤25 kg/m^2 (mean 143.7 ng/mL, s.d. 65.5) compared to those >25 kg/m^2 (121.9 ng/mL s.d. 57.6; P=0.03). IGFBP-3 levels were higher in participants taking tamoxifen (4.17 μg/mL s.d. 1.3 *vs.* 3.9 μg/mL s.d. 0.94; P=0.02) but otherwise did not vary by patient characteristics other than race/ethnicity. IGF-1 correlated positively and strongly with IGFBP-3 (r=0.51, P<0.0001).

Table 2 shows the associations between breast cancer-specific and all-cause mortality, and circulating concentrations of IGF-1 and IGFBP-3. We examined the association between IGF-1 stratified by quintile cut-points. For all-cause mortality there was a significant association between IGF-1 and outcome comparing the highest to lowest quintiles (HR 3.10 95% Confidence Intervals (CI) 1.21-7.93 P= 0.02). There was no significant association between elevated levels of IGF-1 and breast cancer-specific mortality comparing extreme quintiles (HR=2.49 95% CI 0.65-9.51 P=0.18). Exclusion of one outlier had no effect on the results (data not shown).

Levels of IGFBP-3 in the highest quintile were not significantly associated with either reduced risk of all-cause mortality (HR=0.56 95% CI 0.23-1.38 P=0.21), or for breast-cancer mortality (HR=0.54 95% CI 0.16-1.86 P=0.32), for the upper quintile compared to the lowest. There was no association for an interaction term between IGF-1 and IGFBP-3 and either endpoint (data not shown). When categorized using quintile cut-points (table 3), the IGF-1/IGFBP-3 ratio was significantly associated with an increased risk of all-cause

mortality (HR=2.83 95% CI 1.26-6.37, P=0.01; upper vs. lower quintile), but there was no significant association with breast cancer-specific mortality (HR=1.74 95% CI 0.56-5.40 P=0.34; upper vs. lowest quintile).

When we restricted the analysis to women without a diagnosis of diabetes, there was no effect on results for either endpoint (data not shown). Although HOMA scores are associated with survival in this cohort,[1] adjustment of the model by either serum insulin levels or HOMA scores had no effect on the results (data not shown). When we restricted the analysis to postmenopausal women (N=482), levels of IGF-1 (HR=2.20 95% CI 0.79-6.08; upper quintile vs. lower) were not significantly associated with all-cause mortality

We next analyzed the same endpoints for IGF-1 and IGFBP-3 in subgroups of the cohort, using a fully adjusted model. As described above, we used dichotomous variables comparing levels in the highest quintiles of IGF-1 and IGFBP-3, to quintiles 1-4 combined. For participants with a BMI≥25 kg/m^2, increasing levels of IGF-1 were associated with an approximate 2-fold greater increased risk of breast cancer-specific mortality compared to those with a BMI<25 (Table 4), although the test for interaction was non-significant. There was also a 2-fold greater increase in the HR in participants with stage 3 tumors compared to stage 2; again the test for interaction was non-significant. Similar results were observed for all-cause mortality. There was no evidence of effect modification for other subgroups examined.

Discussion

In this study, IGF-1 measured from fasting serum collected from breast cancer survivors approximately 30 months post-diagnosis, was significantly associated with an increased risk of all-cause mortality in a model comparing extreme quintile levels adjusted for treatment received at diagnosis, tamoxifen use at blood draw, race/ethnicity/study-site, IGFBP-3 levels and BMI. Breast cancer specific mortality was not associated with IGF-1. IGFBP-3 was not associated with either all-cause or breast cancer-specific mortality. Restricting the analysis to postmenopausal women or inclusion of menopausal status as a covariate in the model had no effect on the results. The ratio of IGF-1/IGFBP-3 was statistically significantly associated with all-cause mortality for women with the highest ratio (again, in the upper quintile compared to the lowest), which may be interpreted as an indicator of the concentration of free IGF-I. The association between outcome and both IGF-1 and IGF-1/IGFBP-3 levels in our study, was limited to patients with levels of IGF-1 or IGF-1/IGFBP-3 in the highest quintile, suggesting a threshold effect. A pooled analysis[19] and a meta-analysis[30] of the association between breast cancer risk and IGF-1 also reported associations between risk and IGF-1 levels in the highest quintiles and quartiles respectively, compared to the lowest levels of IGF-1.

However one difference was seen in the analysis of IGF-1, where the fourth quintile had a HR =0.7, while the IGF-1/IGFBP-3 ratio, an indicator of free IGF-1 levels, was similar in the second, third and fourth quintiles, and elevated in the fifth. We hypothesized that at high levels of IGF-1 insufficient IGFBP-3 may be produced to bind all of the IGF-1. Excessive IGF-I bioavailability may have the ability to overstimulate cellular growth and IGF-signaling, leading to conditions favorable to tumor growth. [31]

Data on circulating IGF-1 and its association with breast cancer and all-cause mortality in breast cancer survivors are limited. [2, 22, 23, 32] One study reported that the risk of recurrence in 110 postmenopausal breast cancer patients was higher in the fourth quartile (IGF >212.5 ng/mL) compared to the first (IGF <139.2 ng/mL), but this was not statistically

significant; [22] another reported that lower levels of IGF-1 were associated with improved survival in 130 African-American/Hispanic breast cancer patients, although this study was based on both pre- and post-menopausal women.[32] Results from the WHEL study reported that there was no association between IGF-1 levels and recurrence, but WHEL enrolled patients many years after diagnosis, and survival data were not presented, so is not comparable to the present study. [33] Finally, Goodwin *et al.* found no association between IGF-1 levels in 512 women without diabetes, and either distant recurrence or death.[23] In a subsequent analysis with the same data, they reported a tendency for higher levels of IGF-1 to be associated with distant recurrence in a univariate analysis, but this effect was not significant..[23]

In our study, IGFBP-3 was not significantly associated with risk of all-cause mortality. Similarly, Goodwin *et al.* reported that while high levels of IGFBP-3 predicted distant recurrence (upper vs. lower quartile HR=1.8, 95% CI 1.1-3.0), it was not associated with mortality.

As reported by other studies,[34] the association between IGF-1 and BMI was highest in participants with a BMI≤25 kg/m^2, compared to participants with a BMI> 25 kg/m^2. In addition, increasing IGF-1 levels were significantly associated with risk of breast cancer-specific mortality in participants with a BMI>25 kg/m^2, but not in leaner women. A recent study reported an obesity-related gene signature in breast tumors stratified by patients' obesity phenotype. [35] A previously described elevated IGF-1 gene signature [5] was also observed in the obese tumor group, which correlated with the higher obesity signature gene scores, and was associated with both ER-negative breast cancer, and with poor prognosis.[35] The prognostic effect of IGF-1 only in our subset of overweight women, may reflect the presence of these IGF-1 and obesity-related gene signatures.

We saw no evidence of an interaction with tamoxifen in this study. This is surprising, as tamoxifen is known to down-regulate IGF-1 with a number of randomized controlled trials (RCTs) reporting a significant decrease in IGF-1 after tamoxifen treatment. [36, 37, 38, 39] One aspect of current thinking within the insulin-IGF hypothesis is that chronic hyperinsulinemia decreases concentrations of IGF binding proteins leading to increased bioavailable or free IGF-1 with concomitant changes in the cellular environment favoring tumor formation. In our study, the association of IGF-1/IGFBP-3 with outcome was independent of insulin levels, and HOMA scores. IGF-1 also activates gene transcription programs strongly associated with poor breast cancer prognosis.[5, 35]

Our study has several strengths. It is a multi-ethnic population-based cohort of women with incident breast cancer; thus, results are likely to be applicable to the general population. Blood was collected after primary treatment (other than tamoxifen) was completed, and therefore reflects ongoing host factors that can affect prognosis and is not confounded by some concurrent treatments that may influence metabolic markers. We have detailed data on biomarkers, and demographic, anthropometric, tumor, and treatment characteristics, allowing adjustment for a wide range of possible confounders, improving validity.

Our study also has important limitations. First we collected only one measurement of fasting serum and therefore cannot completely characterize the women's exposure to IGF-1 and IGFBP-3. The cohort was established before some current treatments such as aromatase inhibitors and trastuzumab were available, and therefore we cannot estimate what associations IGF1 or IGFBP-3 might have with survival in women using these treatments. Finally, our relatively small numbers of deaths does not allow accurate assessment of risk in specific subcategories such as premenopausal women.

In summary, high levels of IGF-1 were associated with risk of all-cause, but not breast cancer-specific mortality in this cohort of women diagnosed with breast cancer. These results need to be confirmed in larger cohorts of breast cancer survivors with greater numbers of outcomes to provide sufficient power to determine significance of associations.

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Abbreviations

IGF-1	Insulin like Growth Factor-1
IGFBP-3	Insulin like Growth Factor Binding Protein-3
HEAL	Health, Eating, Activity, and Lifestyle Study
SEER	Surveillance, Epidemiology, and End Results
HR	Hazard Ratio
CI	Confidence Interval

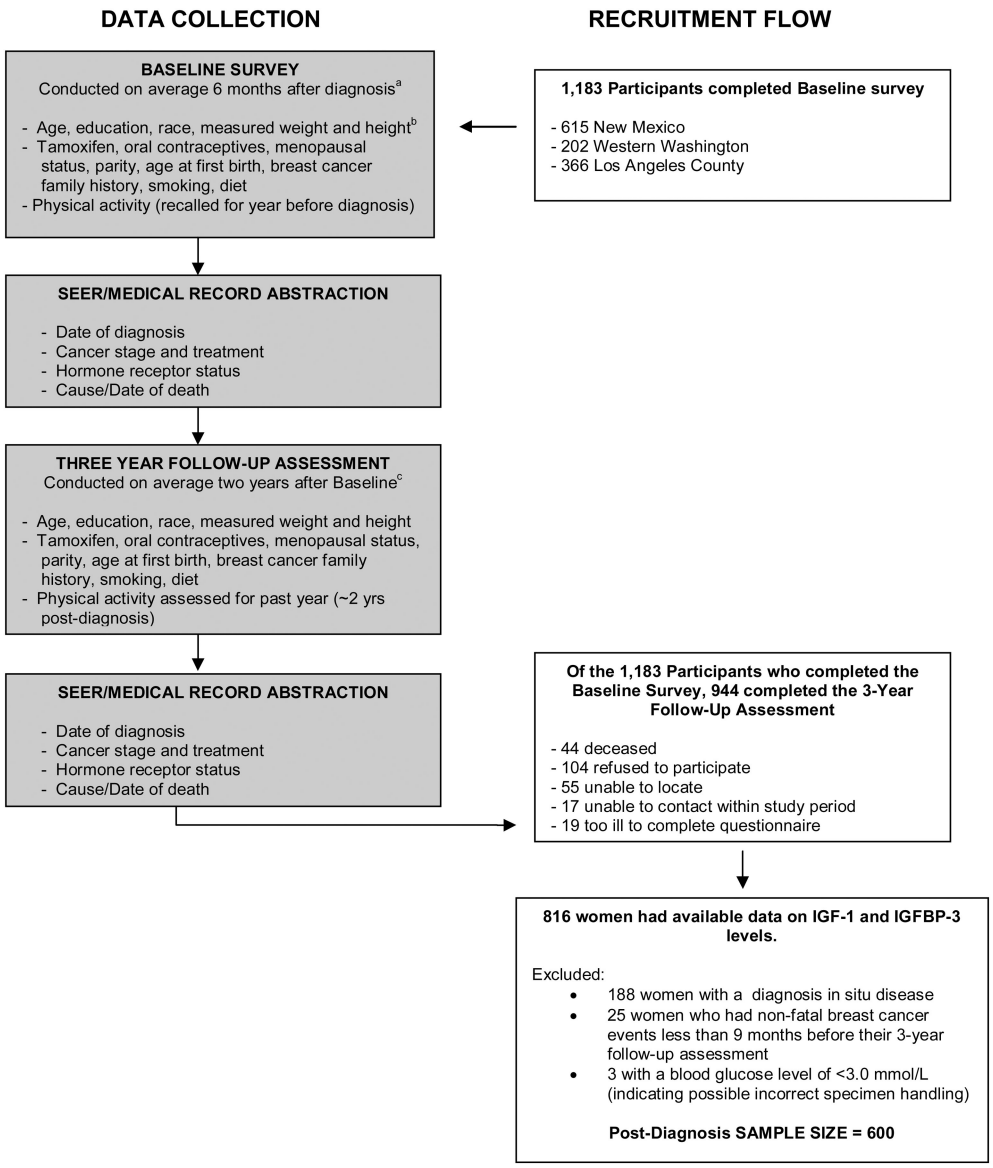

Figure 1. Participant Recruitment and Timing of Data Collection

Table 1
Characteristics of the HEAL cohort N=600

	All[a] N(%)	Non-Hispanic White (NHW) N	African American (AA) N	Hispanic (Hisp.) N
	600	**359 (59.8)**	**155 (25.8)**	**69 (11.5)**
Study Site				
Western Washington	113	97	0	2
New Mexico	332	262	0	67
Los Angeles	155	0	155	0
IGF-1 (ng/mL)				
Mean (Std. Deviation)	130.6 (61.7)	139.7 (56.7)	104.2 (62.38)	146.4 (70.7)
Median	120.6	131.0	93.8	140.0
Range (IQR)	20.0-619.3	36.0-354.0	27.1-619.3	20.0-400.0
IGFBP-3 (μg/ml)				
Mean (Std. Deviation)	4.09 (0.95)	4.20 (0.93)	3.89 (0.99)	4.04 (0.86)
Median	4.08	4.14	3.90	4.16
Range	1.13-6.80	1.68-6.71	1.50-6.80	1.13-5.81
Body mass index (BMI) (kg/m^2)				
Mean (s.d)	27.9 (6.4)	26.7 (5.7)	30.8 (7.5)	27.4 (4. 9)
Median	26.8	25.5	29.6	27.1
Range	16.2-53.5	16.3-48.5	16.2-53.3	18.9-41.0
Age at blood draw				
Mean	57.6 (10.8)	60.4 (11.0)	52.4 (7.9)	56.0 (11.4)
Median	56.5	59.0	52.0	53.0
Range	31.0-89.0	37.0-89.0	38.0-67.0	31.0-83.0
Menopausal Status at blood draw				
Premenopausal	94	49	28	16
Postmenopausal	482	305	111	51
Unknown	24	5	16	2
Estrogen receptor (ER) status				
Negative	117	47	53	16
Positive	425	281	90	40
Unknown	58	31	12	13
SEER[b]summary stage				
Local	428	276	88	53
Regional	172	83	67	16
Treatment at diagnosis				
Surgery	140	80	39	19
Surgery and	221	153	37	24

	All[a] N(%)	Non-Hispanic White (NHW) N	African American (AA) N	Hispanic (Hisp.) N
	600	**359 (59.8)**	**155 (25.8)**	**69 (11.5)**
radiotherapy				
Any chemotherapy	239	126	79	26
Tamoxifen use at blood draw				
No	287	155	86	40
Yes	310	201	69	29
Unknown	3	3	0	0

[a]Surveillance, Epidemiology and End Results (SEER)

Table 2

Associations between IGF-1 and IGFBP-3 and breast-cancer and all-cause mortality

	Events /N total	Unadjusted			Model 1[a]			Model 2[b]		
		HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Breast Cancer Mortality										
IGF-1 (ng/ml) Quintiles										
20.0-79.0	14/120	1.00	Ref.	Ref.	1.00	Ref.	Ref.	1.00	Ref.	Ref.
79.0-109.0	8/124	0.50	0.21-1.21	0.13	0.62	0.24-1.56	0.31	0.69	0.26-1.81	0.45
109.0-136.0	6/118	0.39	0.15-1.04	0.06	0.59	0.19-1.78	0.35	0.88	0.27-2.94	0.84
136.0-178.0	2/121	0.13	0.03-0.55	0.01	0.21	0.04-1.06	0.06	0.28	0.06-1.47	0.13
178.0-619.3	12/117	0.76	0.33-1.70	0.50	1.41	0.46-4.32	0.53	2.49	0.65-9.51	0.18
IGFBP-3 (μg/ml) Quintiles										
1.13-3.30	10/126	1.00	Ref.	Ref.	1.00	Ref.	Ref.	1.00	Ref.	Ref.
3.30-3.90	9/122	0.84	0.33-2.06	0.70	1.13	0.43-2.91	0.81	1.15	0.43-3.12	0.78
3.90-4.30	6/114	0.63	0.23-1.75	0.38	0.63	0.21-1.84	0.40	0.61	0.20-1.93	0.40
4.30-4.90	11/124	0.99	0.42-2.37	0.99	1.34	0.53-3.44	0.54	1.36	0.49-3.81	0.56
4.90-6.80	6/114	0.61	0.21-1.69	0.34	0.67	0.23-1.96	0.47	0.54	0.16-1.86	0.32
All-cause Mortality										
IGF-1 (ng/ml) Quintiles										
20.0-79.0	28/120	1.00	Ref.	Ref	1.00	Ref.	Ref	1.00	Ref.	Ref
79.0-109.0	18/124	0.74	0.40-1.37	0.34	0.93	0.49-1.76	0.82	1.07	0.54-2.08	0.84
109.0-136.0	12/118	0.53	0.26-1.06	0.08	0.73	0.34-1.59	0.43	0.95	0.41-2.17	0.91
136.0-178.0	8/121	0.37	0.16-10.82	0.02	0.53	0.22-1.28	0.16	0.70	0.27-1.81	0.46
178.0-619.3	21/117	1.11	0.61-2.04	0.72	1.87	0.87-4.00	0.11	3.10	1.21-7.93	0.02
IGFBP-3 (μg/ml) Quintiles										
1.13-3.30	28/126	1.00	Ref.	Ref.	1.00	Ref.	Ref.	1.00	Ref.	Ref.
3.30-3.90	15/122	0.71	0.37-1.36	0.31	0.88	0.45-1.73	0.73	0.89	0.44-1.78	0.74
3.90-4.30	15/114	0.82	0.43-1.59	0.56	0.88	0.44-1.74	0.71	0.81	0.38-1.70	0.58

	Events /N total	Unadjusted			Model 1[a]			Model 2[b]		
		HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
4.30-4.90	18/124	0.89	0.48-1.68	0.74	1.15	0.59-2.22	0.67	0.97	0.45-2.10	0.95
4.90-6.80	11/114	0.67	0.32-1.40	0.29	0.78	0.36-1.67	0.52	0.56	0.23-1.38	0.21

[a] Adjusted for **BMI** (categorical <18.5; ≥ 18.5 and < 25; ≥ 25 and < 40; > 40 kg/m^2); **Ethnicity/ Study Site; tamoxifen use at time of blood draw** (Yes/No); **Treatment received at diagnosis** (Surgery/ Radiotherapy+Surgery/Chemotherapy)

[b] This **model contains both IGF-1 and IGFBP-3**, and is adjusted for BMI (categorical <18.5; ≥ 18.5 and < 25; ≥ 25 and < 40; > 40 kg/m^2); Ethnicity/Study Site; tamoxifen use at time of blood draw (Yes/ No); Treatment received at diagnosis (Surgery/Radiotherapy+Surgery/Chemotherapy)

Table 3

Associations between the IGF-1/ IGFBP-3 ratio and breast-cancer and all-cause mortality

	Events /N total	Unadjusted			Model 1[a]		
		HR	95% CI	P	HR	95% CI	P
Breast cancer Mortality							
IGF-1/IGFBP-3 Quintiles (×10^{-3})							
9.35-20.74	11/120	1.00	Ref.	Ref.	1.00	Ref.	Ref.
20.76-27.29	8/120	0.71	0.29-1.78	0.47	0.86	0.33-2.23	0.76
27.30-33.57	7/120	0.61	0.23-1.58	0.31	0.98	0.34-2.84	0.97
33.60-41.45	5/120	0.43	0.15-1.24	0.12	0.83	0.25-2.80	0.77
41.47-91.07	11/120	0.94	0.39-2.25	0.89	1.74	0.56-5.40	0.34
All-cause Mortality							
IGF-1/IGFBP-3 Quintiles (×10^{-3})							
9.35-20.74	19/120	1.00	Ref.	Ref.	1.00	Ref.	Ref.
20.76-27.29	20/120	1.39	0.72-2.68	0.32	1.63	0.83-3.19	0.15
27.30-33.57	14/120	0.93	0.46-1.90	0.84	1.34	0.63-2.88	0.44
33.60-41.45	14/120	0.88	0.44-1.81	0.74	1.33	0.60-2.99	0.47
41.47-91.07	20/120	1.64	0.83-3.21	0.15	2.83	1.26-6.36	0.01

[a]Adjusted for **BMI** (categorical <18.5; ≥ 18.5 and < 25; ≥ 25 and < 40; > 40 kg/m^2); **Ethnicity/ Study Site; tamoxifen use at time of blood draw** (Yes/No); **Treatment received at diagnosis** (Surgery/ Radiotherapy+Surgery/Chemotherapy)

Table 4

Hazard Ratios (HR) and 95% CI of all-cause and breast-cancer specific mortality in subgroups of HEAL participants

	IGF-1[a]			IGFBP-3[b]		
Subgroup	**HR**	**95% CI**	**p[d]**	**HR**	**95% CI**	**p[d]**
Breast-Cancer Specific Mortality[c]						
SEER Stage 2 (Local)	1.00 2.54	Ref 0.82-7.83	0.15	1.00 0.19	Ref 0.03-1.45	0.19
SEER Stage 3 (Regional)	1.00 4.57	Ref 1.48-14.03		1.00 0.83	Ref 0.26-2.59	
ER Negative[e]	1.00 4.74	Ref 1.30-17.29	0.59	1.00 0.71	Ref 0.15-3.38	0.69
ER Positive	1.00 3.33	Ref 1.09-10.17		1.00 0.50	Ref 0.16-1.65	
BMI ≤ 25	1.00 2.17	Ref 0.78-6.03	0.09	1.00 0.46	Ref 0.13-1.768	0.50
BMI >25	1.00 5.82	Ref 1.80-18.83		1.00 0.58	Ref 0.16-2.13	
Tamoxifen at blood draw (No)	1.00 3.40	Ref 1.15-10.11	0.86	1.00 0.67	Ref 0.15-3.04	0.76
Tamoxifen at blood draw (Yes)[f]	1.00 4.01	Ref 1.12-14.43		1.00 0.41	Ref 0.12-1.38	
All-cause Mortality						
SEER Stage 2 (Local)	1.00 2.09	Ref 0.96-4.56	0.05	1.00 0.29	Ref 0.09-0.95	0.11
SEER Stage 3 (Regional)	1.00 4.88	Ref 2.01-11.88		1.00 0.87	Ref 0.32-2.31	
ER Negative[e]	1.00 4.09	Ref 1.44-11.61	0.43	1.00 0.53	Ref 0.11-2.39	0.61
ER Positive	1.00 2.57	Ref 1.19-5.55		1.00 0.62	Ref 0.27-1.41	
BMI ≤ 25	1.00 2.12	Ref 1.00-4.92	0.22	1.00 0.56	Ref 0.21-1.52	0.53
BMI >25	1.00 3.83	Ref 1.70-8.65		1.00 0.52	Ref 0.19-1.40	
Tamoxifen at blood draw (No)[f]	1.00 2.39	Ref 1.12-5.07	0.31	1.00 0.78	Ref 0.29-2.09	0.29
Tamoxifen at blood draw (Yes)	1.00 5.17	Ref 2.03-13.17		1.00 0.33	Ref 0.11-10.93	

[a] IGF-1 comparing levels in the highest quintile to levels in all other quintiles combined

[b] IGFBP-3 comparing levels in the highest quintile to levels in all other quintiles combined

[c] **Fully adjusted model:** IGF-1 and IGFBP-3, BMI (categorical <18.5; ≥ 18.5 and < 25; ≥ 25 and < 40; > 40 kg/m^2); Race/ethnicity/site; tamoxifen use at time of blood draw (Yes/No); treatment received at diagnosis

[d] Test for interaction

[e] 57 (8 deaths; 3 breast cancer deaths) were of unknown ER status

[f] 3 participants had unknown tamoxifen use at time of blood draw.