IGF1 and risk of additional breast cancer in the WHEL study

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Abstract

IGF1, IGF-binding protein-3 (IGFBP-3), IGFBP-1, insulin, leptin, and adiponectin have been inconsistently associated with breast cancer incidence. We explore how these factors are related to breast cancer recurrence and how tamoxifen treatment is related to IGF1 levels among breast cancer survivors in the Women’s Healthy Eating and Living (WHEL) study. A nested case–control design was used to match breast cancer cases (who had an additional breast cancer event) to controls. Baseline blood samples from 510 matched cases and controls were analyzed for IGF1 levels; a subset of 188 pairs were analyzed for five other hormones and binding proteins. Median follow-up was 7.3 years. Matching was on recruitment site, cancer stage, age at cancer diagnosis, dates of cancer diagnosis, and randomization. Cox proportional hazards regression models, stratified on case–control pair, were used to assess the associations. Insulin, IGFBP-1, IGFBP-3, leptin, and adiponectin did not significantly predict recurrence of breast cancer. IGF1 was positively, but not significantly, associated with recurrence (hazard ratio (HR): 1.33 (95% confidence interval (CI) 0.98–1.81)) in the unadjusted analyses. Adjusting for menopausal status and tamoxifen use attenuated the HR to 1.07 (95% CI 0.76–1.40). Analyses of case–control pairs with discordant tamoxifen use show opposing HR: IGF1 predicts higher risk of recurrence if cases did not receive tamoxifen treatment. In conclusion, no significant association was found between IGF1 levels, or other related factors, and risk of additional breast cancer among breast cancer survivors. Tamoxifen can confound analysis of IGF1 and recurrence. This supports re-evaluating significance of IGF1 to breast cancer recurrence.

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Introduction

As a result of early screening and detection and better treatment options, the number of breast cancer survivors is increasing (ACS 2005). However, there remains an ongoing risk of recurrence among a large proportion of survivors (Early Breast Cancer Trialists’ Collaborative Group 1998). The ability to predict women at higher risk of recurrence can help provide patients with new therapies that may improve outcomes. Insulin-like growth factors (IGFs), and more specifically IGF1, have been linked to cell proliferation and growth, as well as cellular transformation and mammary carcinogenesis (Hadsell et al. 2000). IGF-binding proteins (IGFBPs) have an opposite influence to IGF1 on outcomes by limiting the availability of circulating IGF1, as well as directly inhibiting cell growth (Rajaram et al. 1997).

Results of studies of the relationship between IGF1 and breast cancer incidence have been inconsistent. Some studies concluded that higher IGF1 is associated with breast cancer risk only among premenopausal women (Renehan et al. 2004), while other studies reported no association between plasma IGF1 and risk for breast cancer, regardless of menopausal status...
(Kaaks et al. 2002). More recently, new evidence from the European Prospective Investigation into Cancer and Nutrition (EPIC) suggested a positive association between IGF1 and breast cancer incidence only among postmenopausal women (Rinaldi et al. 2006). IGF1 was not associated with recurrence or death from breast cancer in another study of 76 breast cancer recurrences in a cohort of 510 breast cancer survivors (Goodwin et al. 2002). IGF1 was associated with an increased risk of recurrence in the presence of high platelet-derived growth factors in another study of 110 postmenopausal women in Italy (Pasanisi et al. 2008). Vadgama et al. (1999) also found a positive association of IGF1 and breast cancer recurrence among premenopausal African American women.

IGF1 is part of an inter-related metabolic system that includes insulin and two adipocytokines (leptin and adiponectin) that are closely related with insulin (Fischer et al. 2002, Matsubara et al. 2002, Stefan et al. 2002, Wauters et al. 2003). It has been shown that insulin has a role in breast cancer incidence through stimulation of proliferation of breast cancer cells (Milazzo et al. 1992, Mayer et al. 2008). Insulin is central to regulation of IGF1 as well (Straus 1994, Thissen et al. 1994). Insulin was also found to predict recurrence of breast cancer (Goodwin et al. 2002), and in other studies, women with breast cancer and diabetes had a 40% increase in mortality within 5 years following diagnosis compared to women with breast cancer without diabetes (Lipscombe et al. 2008); and breast cancer survivors with HbA1c ≥7.0% had a hazard ratio (HR) of 2.35; 95% CI, 1.56–3.54 in developing a second breast cancer compared with women with HbA1c <6.5% (Petridou et al. 2000, Erickson et al. 2010).

Both adiponectin and leptin have been suggested to be associated with breast cancer incidence (Petridou et al. 2000, Tessitore et al. 2000, Korner et al. 2007, Tworoger et al. 2007). Adiponectin has been found in two studies to be inversely related to breast cancer risk independent of insulin, IGF1, or leptin (Petridou et al. 2000, Miyoshi et al. 2003, Mantzoros et al. 2004). Leptin was not associated with breast cancer risk in one study (Mantzoros et al. 2004), but another study found leptin levels to be inversely related to breast cancer risk (Petridou et al. 2000). To our knowledge, no studies have been published on breast cancer recurrence, which have presented all of these variables. Cancer cells also produce some of these hormones, including IGF1, during breast cancer progression (Macaulay 1992). Therefore, it is unclear whether levels of these hormones are the by-products of carcinogenesis or the early predictors of cancer recurrence. A cohort study design is needed to make such a differentiation prospectively. If these hormones act as early predictors, their routine measurement in breast cancer survivors could identify patients at highest risk who could benefit from additional therapies.

The aims of this analysis were to examine whether baseline circulating concentrations of IGF1 were associated with additional breast cancer events (recurrence or new breast primary) in Women’s Healthy Eating and Living (WHEL) study participants. Additionally, in a subsample, insulin, IGFBP-1, IGFBP-3, as well as adiponectin and leptin were analyzed and assessed for relation to additional breast cancer risk.

Methods
Study participants

The WHEL study was a multi-site, randomized trial that tested the effect of an intensive dietary intervention on disease-free survival in a cohort of women previously diagnosed (within 4 years of their baseline clinic visit) with breast cancer stages I, II, and III (T1–T3, lymph node involvement N0–N3, but without metastasis (M0) breast cancer; Pierce et al. 2002). They had to be between the ages of 18 and 70 years at the time of diagnosis. All participants were recruited between 1995 and 2000 from one of seven clinical sites in California, Arizona, Texas, and Oregon. In the 6 months prior to enrollment, all participants were deemed cancer free by an oncologist. Participants were randomly allocated to either an intervention or comparison group and followed up for a median of 7.3 years. The intervention group was encouraged to adopt a plant-based, low-fat dietary pattern through a telephone counseling protocol (Newman et al. 2005). Comparison group participants were advised to follow National Cancer Institute dietary guidelines, and were provided with print materials from the US Department of Agriculture and the National Cancer Institute but did not receive individualized counseling (Pierce et al. 2002).

Characteristics of original breast cancer diagnosis and treatment (date of diagnosis, type of surgery, tumor stage, grade, estrogen receptor status, and adjuvant chemotherapy) were extracted from medical records and confirmed by an oncologist, with a random sample of records reviewed by a second oncologist. Participants were contacted by study staff twice annually between recruitment and study end in 2006 to report on any new cancers. Medical records for each reported breast cancer recurrence or new primary breast cancer...
diagnosed after study enrollment were reviewed and confirmed by two oncologists.

Demographic variables, dietary intake, measures of height and weight, and changes in health symptoms were collected by telephone and during clinic visits at baseline and during follow-up years. Physical activity level (metabolic equivalent tasks = MET minutes per week) was computed from the Personal Habits questionnaire originally developed for the Women’s Health Initiative (WHI) clinical trial and validated on the WHEL sample (Johnson-Kozlow et al. 2007). Fasting blood samples were collected at enrollment, and serum and plasma aliquots were stored at the WHEL coordinating center in San Diego, CA, USA.

A total of 510 breast cancer cases (those who developed a second breast cancer as a recurrence or a primary cancer and hereafter referred to as a recurrence) and matched pairs were analyzed for IGF1 baseline (i.e. study entry) levels. Owing to budgetary restrictions, only 188 samples from recurrent or new primary breast cancer cases and their matched controls were additionally analyzed for baseline insulin, IGFBP-1, IGFBP-3, leptin, and adiponectin. Institutional review boards for all participating institutions approved the procedures for this study, and informed consent was obtained from all study participants.

**Study design**

For this analysis, each woman with a recurrent/new primary breast cancer event (case) in the WHEL cohort was matched using a nested case–control design with someone (control) who was disease-free at the time that the case was diagnosed. This matching strategy follows the work of Lubin & Gail (1984) and Langholz & Thomas (1990). Controls could be matched with more than one case, and women who subsequently recurred could be controls for women who recurred earlier. Matching criteria were clinical site, cancer stage (American Joint Committee on Cancer 2002), age at cancer diagnosis, and dates of cancer diagnosis and randomization into the WHEL study. Most matched pairs (67% for date of diagnosis and 88% for date randomized) were concordant within 12 months of each other, and virtually all matched pairs were concordant within 3 years.

**Laboratory analysis**

Fasting blood samples were collected from participants during the clinic visits, immediately placed on ice, protected from light, and separated within 1 h after collection, using centrifugation at 2300 g at 4 °C for 10 min. Vials were frozen for at least 2 h in −70 °C freezer before being packaged and shipped. Samples were shipped with dry ice to the coordinating center at the University of California, San Diego for storage at −70 °C or lower. Serum aliquots were analyzed for levels of IGF1 and IGFBP-3 at the University of Southern California Reproductive Endocrine Research Laboratory using the DPC Immulite Immunology analyzer. IGFBP-1 was analyzed with Diagnostics System Laboratories (Webster, TX, USA) IRMA (IRMA kit DSL-7800). The assay sensitivity and inter-assay coefficient of variations (CV) were: IGF1 sensitivity of 20 ng/ml with a CV% <7.7; IGFBP-1 sensitivity of 0.33 ng/ml and CV% <7.0, and IGFBP-3 sensitivity of 100 ng/ml and CV% <5.5.

Leptin, insulin, and adiponectin were measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont, using the Luminex Technology, ELISA, and LINCOplex kits. Adiponectin was measured by ELISA Quantikine kit (R&D Systems, Minneapolis, MN, USA). Inter-assay CV% for adiponectin was 6.8–12.2. Human Serum Adipokine Panel B LINCOplex kit (Linco Research, Inc., St Charles, MO, USA) measured leptin and insulin. The assay ranges for each analyte were 16–250 000 and 3.2–50 000 pg/ml respectively. Inter-assay CV were 8.0–9.9 for leptin and 5.5–14.3 for insulin. Owing to insufficient plasma aliquot volume, nine of the 345 assays were not performed for leptin and insulin, and ten assays had missing data for adiponectin.

**Statistical analysis**

Descriptive statistics were used to compare women who had a subsequent breast cancer event (recurrence or new primary breast cancer) with their matched controls, using paired t-tests for continuous variables and McNemar’s paired χ² tests for categorical variables. For the latter test, tumor grade variable was binarized into grade I versus other (grades II, III and unspecified). Hormone concentrations were tested for normality with the Shapiro–Wilk statistic, and all variables were log transformed to improve normality. We report median (interquartile range) as well as means and S.E.M. We also selected quartile cut-off points in non-recurred controls to identify any threshold effect of IGF1 on the risk of recurrence (a second breast cancer).

For the analytes insulin, IGFBP-1, IGFBP-3, leptin, and adiponectin, 188 pairs were used. For IGF1, laboratory analysis results were available for 510 cases and their matched controls. Analyses included Cox proportional hazards regression models stratified by pair number to examine the relationship between
hormone levels and recurrence-free survival (Langholz & Thomas 1990). The stratified Cox proportional hazards models are equivalent to conditional logistic regression (Goldstein & Langholz 1992, Bernstein et al. 2004). The models were adjusted for potential confounders. Stage and site were not included in survival models because all pairs were matched exactly on these variables. Notably, age and time between diagnosis and study entry were matched but differed statistically between cases and controls by 2–3 months. Because this is not a meaningful difference and to develop parsimonious models, these variables were not included in the model. Other variables not included as matching criteria were tumor grade, type of surgery, tamoxifen therapy at baseline, estrogen receptor status, adjuvant chemotherapy, body mass index, physical activity, and menopausal status (defined as amenorrheic for 12 months) and were included in the Cox models only if they significantly differed between cases and controls. For IGFI, we also analyzed association with recurrence in a subset of matched pairs of cases and controls where both members of the pair were postmenopausal (N=344 pairs).

Finally, we re-ran the models on subgroups matched for tamoxifen use in the full (N=284 matching pairs and N=226 discrepant pairs) and postmenopausal sample (N=195 matching pairs and N=149 discrepant pairs) because IGFI levels were lower among tamoxifen users.

### Results

We assessed between group differences in tumor characteristics at initial diagnosis, and treatment by case-control status. No significant difference was observed between the two groups in tumor stage because this was a matching variable. The observed statistically significant differences between cases and controls on age and time from diagnosis to randomization were <3 months, and hence not meaningfully different. Menopausal status and tamoxifen use were the only variables that were statistically different between cases and controls, but distributions of other variables (i.e. type of surgery, use of chemotherapy, body mass index (kg/m²) or physical activity) were similar between cases and controls (Table 1).

Insulin, IGFBP-1, IGFBP-3, leptin, and adiponectin did not differ significantly by breast cancer status (Table 2). Similarly, none of the HRs for these hormones were statistically significant in predicting a second breast cancer event in the multivariate Cox proportional hazards model adjusting for tamoxifen use. In particular, IGFBP-3 (HR 0.81, 95% CI 0.33–1.99) and insulin (HR 0.81, 95% CI 0.55–1.19) had HRs below one but were not statistically significant. In the 188 pairs with both IGFI and IGFBP-3 measures, the HR for log transformed IGFI on recurrence (adjusted for tamoxifen) was 1.20, 95% CI (0.68–2.12). Further adjustment for IGFBP-3

| Table 1 Breast cancer risk factors among recurred cases and matched non-recurred controls among women participating in the WHEL study |
|---------------------------------|-----------------|-----------------|
| **Cases (recurred)**            | **Controls (did not recur)** | **P** |
| **(N=510)**                     | **(N=510)**      |      |
| Tamoxifen use at study entry (%)| 53.1             | 63.7             | 0.0003 |
| Period between diagnosis to randomization (months (mean (s.d.))) | 23.2 (12.2) | 25.0 (118) | 0.0002 |
| Tumor grade (%)                 |                  |                  | 0.12   |
| I                               | 8.2              | 11.2             |
| II                              | 37.7             | 42.6             |
| III                             | 45.3             | 38.4             |
| Unspecified                     | 8.8              | 7.8              |
| Estrogen receptor status (%)    |                  |                  | 0.10   |
| +                               | 70.3             | 74.9             |
| -                               | 29.7             | 25.1             |
| Underwent chemotherapy (%)      | 80.2             | 80.6             | 0.84   |
| Had mastectomy (%)              | 58.9             | 60.2             | 0.63   |
| Body mass index (mean (s.d.))   | 27.6 (6.2)       | 27.7 (5.8)       | 0.80   |
| Physical activity MET-min/weeks (mean (s.d.)) | 796 (827) | 774 (790) | 0.62   |
| Age in years (mean (s.d.))      | 51.1 (9.6)       | 51.4 (9.0)       | 0.01   |
| Menopausal status (%)           |                  |                  | 0.01   |
| Postmenopausal                  | 74.7             | 80.2             |
| Premenopausal                   | 25.3             | 19.8             |

*Paired t-test used for continuous variables, and McNemar’s paired $\chi^2$ test for binarized categorical variables (tumor grade binarized into stage I versus the rest of the stages).
strengthened the IGF1 HR to 1.41 but did not reach statistical significance (95% CI for HR was 0.72–2.76).

As mentioned earlier, a larger sample of 510 case–control pairs was analyzed for IGF1. In the unadjusted Cox model of the log-transformed IGF1, the HR for recurrence per unit increase of log IGF1 was 1.33 (95% CI 0.98–1.81, \( P=0.06 \)), but adjustment for tamoxifen use and menopausal status markedly reduced this HR to 1.07 (95% CI 0.76–1.40, \( P=0.72 \); Table 3). In our quartile analysis of IGF1 and recurrence, the HRs for the upper three quartiles were not significantly different from the lowest (reference) quartile (quartile 4 HR = 1.21, 95% CI = (0.95–1.54); quartile 3 HR = 1.18, 95% CI = (0.93–1.51); quartile 2 HR = 0.85, 95% CI = (0.65–1.11)). IGF1 levels were higher in cases than controls (mean (S.E.M.) was 114.1 (2.3) vs 108.3 (2.1) ng/ml, \( P=0.06 \)). We found that IGF1 was significantly lower in women who took tamoxifen, regardless of menopausal status (Table 3), so we ran recurrence models stratified by tamoxifen treatment. Tamoxifen use was inversely and significantly related to recurrence \( HR=0.66 \) (95% CI 0.48–0.89) and remained so even after excluding IGF1 from the model. We carried out further analyses for IGF1 by matching cases and controls on tamoxifen treatment status and found 284 pairs whose tamoxifen use matched pairs (185 pairs in which both were on tamoxifen and 99 pairs in which neither were on tamoxifen; Table 3). The association between IGF1 and recurrence became inverse but not statistically significant in the pairs in which both cases and controls either received or did not receive tamoxifen (HR = 0.80, 95% CI 0.47–1.37 for 185 pairs on tamoxifen; HR = 0.96, 95% CI 0.44–2.08 for 99 pairs not on tamoxifen). Furthermore, among cases using tamoxifen and controls not using tamoxifen (discordant pairs), the HR was 0.05 (95% CI 0.01–0.20), whereas in cases not using tamoxifen and controls using tamoxifen, the HR was 18.1 (95% CI 6.84–48.07).

Among 344 postmenopausal case–control pairs, we further analyzed the effects of IGF1 on recurrence to assess whether the results changed in this subpopulation. Mean (S.E.M.) IGF1 among cases was 105.4 (2.7) and 97.8 (2.4) ng/ml in matched controls, \( P=0.03 \). Among postmenopausal women in whom both cases and controls took tamoxifen, the HR for IGF1 and breast cancer recurrence was 0.92 (95% CI 0.52–1.63) and among postmenopausal women in whom both cases and controls were not on tamoxifen, the HR was 0.65 (95% CI 0.20–2.07). However, among cases on tamoxifen and controls not on tamoxifen, the HR was 0.05 (95% CI 0.01–0.24), and conversely in cases not on tamoxifen and controls on tamoxifen, the HR was 51.74 (95% CI 10.87–246.4). Analyses for pre/peri-menopausal women were not undertaken as there were only 64 pairs.

### Discussion

We found no significant association between IGF1, IGFBP-1, IGFBP-3, insulin, adiponectin, or leptin and additional breast cancer events in a cohort of breast cancer survivors. When we matched IGF1 for tamoxifen treatment use, the association became attenuated and closer to the null. Furthermore, when comparing discordant tamoxifen use for pairs of cases and controls, a higher risk of recurrence in relation to high IGF1 levels was found if cases did not receive tamoxifen treatment while controls received it, which supports a major confounding effect of tamoxifen on the association.


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**Table 2** The mean, median/interquartile range (IQR), and hazard ratio for recurrence for insulin, IGF-binding protein-1 (IGFBP-1), IGFBP-3, leptin, and adiponectin in recurred breast cancer cases and their matched controls.

<table>
<thead>
<tr>
<th></th>
<th>Cases (recurred) (N=188)</th>
<th>Controls (non-recurred) (N=188)</th>
<th>Paired t-test (P)</th>
<th>Hazard (95% CI)</th>
<th>( P^a )</th>
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<tbody>
<tr>
<td><strong>Insulin (pg/ml)</strong></td>
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<tr>
<td>Mean (S.E.M.)</td>
<td>320 (16)</td>
<td>354 (23)</td>
<td>0.32</td>
<td>0.81 (0.55–1.19)</td>
<td>0.29</td>
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<tr>
<td>Median (IQR)</td>
<td>247 (196, 364)</td>
<td>272 (200, 414)</td>
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<td><strong>IGFBP-1 (ng/ml)</strong></td>
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<tr>
<td>Mean (S.E.M.)</td>
<td>32.0 (1.5)</td>
<td>33.7 (1.8)</td>
<td>0.80</td>
<td>1.06 (0.81–1.39)</td>
<td>0.66</td>
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<tr>
<td>Median (IQR)</td>
<td>27.5 (15.1, 47.3)</td>
<td>29.7 (13.9, 47.6)</td>
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<td><strong>IGFBP-3 (μg/ml)</strong></td>
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<tr>
<td>Mean (S.E.M.)</td>
<td>4.17 (0.07)</td>
<td>4.21 (0.07)</td>
<td>0.59</td>
<td>0.81 (0.33–1.99)</td>
<td>0.65</td>
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<tr>
<td>Median (IQR)</td>
<td>4.05 (3.60, 4.70)</td>
<td>4.05 (3.60, 4.80)</td>
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<td><strong>Leptin (pg/ml)</strong></td>
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<tr>
<td>Mean (S.E.M.)</td>
<td>23 521 (1573)</td>
<td>23 117 (1369)</td>
<td>0.77</td>
<td>1.03 (0.80–1.32)</td>
<td>0.84</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>18 074 (9583, 3182)</td>
<td>18 777 (9663, 29 315)</td>
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<td><strong>Adiponectin (ng/ml)</strong></td>
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<tr>
<td>Mean (S.E.M.)</td>
<td>9301 (334)</td>
<td>9449 (365)</td>
<td>0.95</td>
<td>1.04 (0.70–1.54)</td>
<td>0.87</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>8403 (5835, 11 887)</td>
<td>8066 (5838, 13 299)</td>
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</table>

Paired t-tests and Cox models are run on log-transformed pair-wise data.

\( a \) Cox proportional hazard models are adjusted for tamoxifen use.
Evidence from the European Prospective Investigation into Cancer and Nutrition (EPIC) suggested a positive association between IGF and breast cancer incidence only among postmenopausal women (Rinaldi et al. 2006), while previous study findings suggested an association only among premenopausal women (Renehan et al. 2004). More recently, a pooled analysis of 17 prospective studies found a positive association between IGF1 and breast cancer incidence regardless of menopausal status (Endogenous Hormones and Breast Cancer Collaborative Group 2010), while results from the Women’s Health Initiative Cohort and the Japan Collaborative Cohort Study for Evaluation of Cancer Risk failed to find an association (Gunter et al. 2009, Sakauchi et al. 2009). The study by Goodwin et al. (2002), one of the few prospective studies that investigated the association of IGF1 and insulin in relation to breast cancer recurrence, reported comparable results and found a non-significant HR for recurrence of breast cancer among women with the highest IGF1 quartile compared with the lowest quartile (1.55 (95% CI 0.98–1.81)). Non-significant results were also found in a more recent study involving recurrence among 110 postmenopausal women (Pasanisi et al. 2008). Furthermore, our results are not inconsistent with a retrospective study of 130 African Americans and Latino breast cancer patients where plasma IGF1 levels were not associated with increased risk of breast cancer recurrence among postmenopausal women (Vadgama et al. 1999), although a significant association was found among premenopausal women.

In a recent trial that investigated the influence of tamoxifen on IGF1 among 235 premenopausal breast cancer women (Decensi et al. 2009), tamoxifen
lowered IGF1 levels by 17%, and was negatively associated with recurrence of breast cancer. IGF1 was not significantly related to recurrence, and the association with recurrence was inverse except for the highest IGF1 tertile. However, only 48 cases of breast cancer recurrence occurred in that study. Findings from our study of a larger sample indirectly support results of the above trial; when we carefully adjusted for tamoxifen, it explained any potential influence of IGF1 on recurrence of breast cancer. In our study, IGF1 did not predict breast cancer recurrence risk among women stratified for tamoxifen use, and the initial positive association of IGF1 became negative when matching for tamoxifen use. To further determine the confounding effect of tamoxifen, when we limited the analyses to controls who were on tamoxifen while their matching cases were not, IGF1 became highly and positively predictive of breast cancer recurrence because its influence was limited to the cases while the controls were taking tamoxifen that blocked any effect of IGF1 on recurrence. Further supporting this argument, when the analyses were limited to cases who were on tamoxifen and their matched controls who were not, IGF1 became inversely associated with breast cancer recurrence because its influence in predicting higher recurrence was among the controls who did not take tamoxifen while any influence on the cases was blocked by the tamoxifen they were taking. This demonstrates that IGF1 has no effect independent of tamoxifen in predicting breast cancer recurrence.

We did not find a significant association between insulin and recurrence of breast cancer. In contrast, Goodwin et al. (2002) found that high insulin levels were associated with a twofold increase in risk of distant recurrence and a threefold increase in risk of death among postmenopausal breast cancer survivors. Several IGFBPs have been identified (Oh 1998), but the most important is IGFBP-3, which binds 95% of IGF1 and has been reported to be secreted by the breast tissue (Figueroa et al. 1993). IGFBP-3 usually acts opposite to IGF1, which it transports in the circulation. There was no change in our results when we used the ratio of IGF1/IGFBP-3 to predict breast cancer (HR of log-transformed IGF1 to log-transformed IGFBP-3 was 0.91, 95% CI 0.68–1.23, P = 0.54). Higher levels of IGFBP-3 are associated with a decreased risk of breast cancer among women (Toniolo et al. 2000, Kaaks et al. 2002, Krajcik et al. 2002, Muti et al. 2002) who are premenopausal (Bruning et al. 1992, Hankinson et al. 1998, Ng et al. 1998, Mantzoros et al. 2004, Rinaldi et al. 2006) as well as postmenopausal (Kaaks et al. 2002, Krajcik et al. 2002, Muti et al. 2002, Keinan-Boker et al. 2003). A study by Goodwin et al. (2002) found that IGFBP-3 was associated with an increased risk of breast cancer recurrence only among postmenopausal women (HR 3.33 (95% CI 1.46–7.63)). The direction of association between IGF1 and IGFBP-3 with breast cancer risk has been inconsistent in prior studies and varied according to menopausal status.

Adiponectin and leptin are two of several adipocytokines (a group of polypeptide growth factors and cytokines mostly produced by white adipose tissue adipocytes). These proteins are secreted by adipocytes as well as by the mammary epithelial cells; circulating adiponectin levels are inversely related, whereas leptin levels are positively related to insulin resistance (Smith-Kirwin et al. 1998, Hotta et al. 2000, Kubota et al. 2002, Matsubara et al. 2002, Stefan et al. 2002). A study that followed 471 breast cancer patients for overall survival and recurrence found no significant association between leptin and survival or recurrence in the multivariate models (Goodwin et al. 2005). Leptin was not associated with breast cancer incidence risk in some studies (Mantzoros et al. 2004, Sauter et al. 2004, Stattin et al. 2004), but inversely related in others (Petridou et al. 2000). Results from adiponectin studies and incidence of breast cancer were also inconsistent, with some studies finding an inverse association (Mantzoros et al. 2004, Tworoger et al. 2007) and other studies finding a positive association (Miyoshi et al. 2003, Korner et al. 2007) with breast cancer risk.

An advantage of our study was the nested case–control design matching for recognized risk factors for breast cancer recurrence. Earlier analyses from the WHEL intervention indicated no influence of the dietary intervention on IGF1 and the related growth factors examined in this analysis (Al-Delaimy et al. 2006). We prospectively collected and verified data on recurrence by medical record review (Pierce et al. 2002). A disadvantage was the limited number of cases analyzed for insulin, leptin, adiponectin, and IGFBP that did not allow us to carry out analyses by menopausal status for factors other than IGF1. Another limitation is that our study participants may underestimate cases of early recurrence because WHEL study participants were recruited up to 4 years after their initial diagnosis.

In conclusion, our results do not support an association between high IGF1 levels and risk of recurrence among postmenopausal women. An initial positive association was confounded by tamoxifen use. Other energy balance-related hormones were also not related to recurrence in our study. The role of
tamoxifen and other anti-estrogens and their influence on the endogenous hormones and their association with recurrence of breast cancer need to be investigated in studies of breast cancer survivors.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References
Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, Ficker K & Julius U 2002 Insulin-resistant patients with type 2 diabetes mellitus have higher


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