Circulating concentrations of insulin-like growth factor I and risk of breast cancer

Susan E Hankinson, Walter C Willett, Graham A Colditz, David J Hunter, Dominique S Michaud, Bonnie Deroo, Bernard Rosner, Frank E Speizer, Michael Pollak

Summary

Background Insulin-like growth factor (IGF)-I, a mitogenic and antiapoptotic peptide, can affect the proliferation of breast epithelial cells, and is thought to have a role in breast cancer. We hypothesised that high circulating IGF-I concentrations would be associated with an increased risk of breast cancer.

Methods We carried out a nested case-control study within the prospective Nurses' Health Study cohort. Plasma concentrations of IGF-I and IGF binding protein 3 (IGFBP-3) were measured in blood samples collected in 1989–90. We identified 397 women who had a diagnosis of breast cancer after this date and 620 age-matched controls. IGF-I concentrations were compared by logistic regression with adjustment for other breast-cancer risk factors.

Findings There was no association between IGF-I concentrations and breast-cancer risk among the whole study group. In postmenopausal women there was no association between IGF-I concentrations and breast-cancer risk (top *vs* bottom quintile of IGF-I, relative risk 0·85 [95% CI 0·53–1·39]). The relative risk of breast cancer among premenopausal women by IGF-I concentration (top *vs* bottom tertile) was 2·33 (1·06–5·16; p for trend 0·08). Among premenopausal women less than 50 years old at the time of blood collection, the relative risk was 4·58 (1·75–12·0; p for trend 0·02). After further adjustment for plasma IGFBP-3 concentrations these relative risks were 2·88 and 7·28, respectively.

Interpretation A positive relation between circulating IGF-I concentration and risk of breast cancer was found among premenopausal but not postmenopausal women. Plasma IGF-I concentrations may be useful in the identification of women at high risk of breast cancer and in the development of risk reduction strategies. Additional larger studies of this association among premenopausal women are needed to provide more precise estimates of effect.

Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School (S E Hankinson ScD, Prof W C Willett MD, G A Colditz MB, D J Hunter MB, D S Michaud BA, Prof B Rosner PhD, F E Speizer MD)**; Departments of Epidemiology** (S E Hankinson, Prof W C Willett, G A Colditz, D J Hunter, D S Michaud) **and Nutrition** (Prof W C Willett, D S Michaud), **Harvard School of Public Health, Boston MA, USA; and Departments of Medicine and Oncology, Cancer Prevention Research Unit, Lady Davis Institute of the Jewish General Hospital and McGill University, Montreal, Quebec, Canada** (B Deroo BSc, M Pollak MD).

Correspondence to: Dr Susan E Hankinson, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA

Introduction

There is substantial experimental evidence that the growth hormone/insulin-like growth factor (IGF)-I axis not only affects the proliferative behaviour of breast cancer,[1–3] but also stimulates proliferation of normal breast epithelial cells.[4] Transgenic mice that overexpress growth hormone have a high frequency of breast cancer, and overexpression of IGF-I-receptor agonists is associated with cancer and incomplete mammary involution.[5–7] Similarly, rhesus monkeys treated with growth hormone or IGF-I have mammary gland hyperplasia.[4] By contrast, ductal hypoplasia is seen in animals that overexpress a growth-hormone antagonist.[8,9] Studies in mice have shown a positive correlation between the strain-specific incidence of breast cancer and the activity of the growth hormone/IGF-I axis.[10]

There is a large variation between individuals in circulating concentrations of IGF-I and IGF binding protein 3 (IGFBP-3)—the principal IGFBP in the circulation.[11,12] The effect of IGF-I on the proliferation of breast epithelial cells and evidence that increased turnover in certain epithelial-cell populations is associated with a greater risk of neoplastic transformation, led us to investigate the relation of circulating IGF-I concentrations and the risk of breast cancer in women.[4,13]

Methods

We carried out a nested case-control study within the Nurses' Health Study cohort. The Nurses' Health Study started in 1976 when 121 700 female registered nurses aged 30–55 years completed and returned a mailed questionnaire. The cohort is assessed every 2 years by questionnaire to update exposure status and to identify newly diagnosed disease. Data have been collected about breast-cancer risk factors such as height, weight, age at menarche, menopause, and at the birth of their first child, postmenopausal hormone use, and family history of breast cancer in mother, sister, or both. As of 1994, the follow-up rate was 90%.

In 1989–90, blood samples were collected from 32 826 women who were any age between 43 and 69 years inclusively. Details of the blood collection methods and storage have been previously published.[14] The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

There is evidence that a single plasma IGF-I measurement, such as we have, reflects longer term circulating IGF-I concentrations: a correlation coefficient of 0·94 was found among 24 adults who had two blood samples drawn about 6 weeks apart.[15]

Cases in this analysis did not have a diagnosis of invasive breast cancer before blood collection but were diagnosed before June 1, 1994. The diagnosis in 398 of 399 women was confirmed by review of the medical records. For one case, the woman confirmed the diagnosis, but the medical record was unavailable. Because of the high confirmation rate (99%) by record review, this case was kept in the analysis.

Menopausal status was defined at the time of blood collection. A woman was defined as premenopausal if she had had at least one natural menstrual cycle in the previous 12 months or was

	Cases (n=397)	Controls (n=620)
Postmenopausal women		
Age (years)	61·2 (5·3)	61·6 (4·9)
Age at menopause (years)	48·8 (4·5)	49·5 (4·0)
Age at menarche (years)	12·4 (1·4)	12·6 (1·5)
Parity*	3·2 (1·5)	3·5 (1·5)
Family history of breast cancer (%)	16·9	12·8
Premenopausal women		
Age (years)	48·1 (2·8)	48·1 (2·8)
Age at menarche (years)	12·6 (3·4)	12·5 (1·4)
Parity*	2·7 (1·0)	2·8 (1·0)
Family history of breast cancer (%)	10·5	8·6

Mean (SD). *Among parous women only.

Table 1: **Baseline characteristics**

	Cases		Controls		p
	n	Median (range)	n	Median (range)	
All cases or controls	397	154 (20·7–425)	620	158 (23·9–464)	0·83
Postmenopausal	305	142 (20·7–390)	483	153 (23·9–464)	0·62
Premenopausal	76	204 (40·1–425)	105	184 (81·0–320)	0·08
Premenopausal, age ≤50	60	206 (77·6–425)	78	175 (84·9–320)	0·009

Table 2: **Plasma IGF-I concentrations by case or control status**

younger than 48 years (if a non-smoker) or than 46 years (if a current smoker) after hysterectomy without bilateral oophorectomy—at these ages less than 10% of the cohort had had a natural menopause. A woman was defined as postmenopausal if she reported a natural menopause or bilateral oophorectomy, or if she was at least 56 years (if a non-smoker) or 54 years (if a current smoker) after hysterectomy without bilateral oophorectomy—by these ages a natural menopause had occurred in 90% of the cohort.

For each case with breast cancer one control was matched by year of birth, time of day blood was drawn, fasting status (more than 10 h after a meal or not), month of blood sampling, menopausal status, and use of postmenopausal hormones at the time of blood collection—since these hormones tend to lower IGF-I concentrations.[16] For each of the 140 postmenopausal cases not on hormone-replacement therapy at blood collection, a second control was selected. A further 89 controls matched to cases with in-situ breast cancer and two controls matched to a case in whom the diagnosis was subsequently disconfirmed were also included. Plasma IGF-I concentrations were not available for two cases and ten controls; thus there were 397 cases and 620 controls in this analysis.

IGF-I and IGFBP-3 were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX, USA). This method is more reproducible and appropriate for large numbers of samples than the RIA previously employed.[2] There was a high correlation between the IGF-I values from the ELISA and samples analysed by RIA after acid chromatography (Pearson r=0·97). All samples were assayed masked to case or control status. Masked split specimens included within each batch were used to calculate the coefficient of variation within and between batches: for IGF-I these were 8·7% and 15·6%, respectively; for IGFBP-3, 9·3% and 19·4%, respectively. To find out the effect of our blood-collection methods on IGF-I concentrations, we compared IGF-I and IGFBP-3 concentrations in samples that were separated and frozen immediately—the usual collection and processing methods—with samples stored as heparinised whole blood for 24 h and 36 h before separation (similar to our collection conditions). The mean IGF-I and IGFBP-3 values were almost identical and the intraclass correlation between results of the two collection methods was 0·98 for IGF-I and 0·96 for IGFBP-3, which showed that our collection methods did not affect sample integrity.

Statistical analyses

We analysed the relation between plasma IGF-I concentration and breast-cancer risk in the whole group and by menopausal status. 16 cases and 32 controls in whom menopausal status was uncertain were excluded from the stratum-specific analyses.

For analyses among all women and postmenopausal women only, quintiles of IGF-I and IGFBP-3 were based on the values from all controls. Because of the smaller number of premenopausal women tertiles were used, based on the values in the premenopausal controls.

IGF-I was assessed in the models with and without IGFBP-3 as a factor since there is evidence that IGFBP-3 may decrease IGF-I bioactivity by direct or indirect mechanisms.[17] In another study the ratio between plasma IGF-I and IGFBP-3

concentrations was highly associated with breast-cancer risk and so this ratio was also assessed.[18]

A linear regression model of IGF-I by case or control status and adjusted for the use of hormone-replacement therapy was used to compare mean IGF-I concentrations between cases and controls. Because of the skewed distribution of the IGF-I values, the square-root was used and we accounted for the correlation between multiple control values per case.[19] Unconditional logistic regression analysis adjusted for the matching factors was used to estimate the relative risks (odds ratios) and 95% CI. Adjustment for other breast-cancer risk factors (age at menarche [less than 12 years, 12 years, 13 years, more than 14 years], age at menopause [premenopausal, less than 44 years, 45–49 years, more than 50 years, unknown], parity [0, 1–2 births, 3–4 births, more than 5 births], age at birth of first child [0, less than 24 years, 25–29 years, 30 years or older], family history of breast cancer, and body-mass index [less than 23·0 kg/m², 23·0–28·9 kg/m², 29·0 kg/m² or greater]) did not substantially alter the relative risk, thus estimates adjusted only for the matching factors are presented here. The results of the unconditional analyses were essentially identical to those from conditional analyses but had the advantage of increased statistical power. Tests for trend were done by calculating the Wald statistic for a continuous distribution of IGF-I values.[20]

Results

Baseline characteristics of the cases and controls, by menopausal status, are shown in table 1. The mean time between blood collection and diagnosis was 28 months (SD 16) with a range of 1–57 months.

There was a significant relation between IGF-I and age (r=-0·12, p=0·01). IGF-I concentrations did not differ between cases and controls in the whole cohort or among postmenopausal women (table 2). Among premenopausal women younger than 50 years, the mean IGF-I concentration was significantly higher in the cases than in the controls (p=0·009).

There was no association between IGF-I concentration and breast-cancer risk among the whole study population after adjustment for the breast-cancer risk factors (table 3). When IGFBP-3 was added to the statistical model, the relative risks were similar. The relative risk of in-situ breast cancer by IGF-I concentration also was not significant (2·26 top *vs* bottom quintile, 95% CI 0·70–7·28).

In postmenopausal women there was no relation between IGF-I and breast-cancer risk even after exclusion of current or recent users of hormone-replacement therapy.

There was a positive association between IGF-I concentration and breast-cancer risk in premenopausal women that increased when tertiles of IGFBP-3 were added to the model (top *vs* bottom tertile of IGF-I, relative risk 2·88 [1·21–6·85]). The relative risk was 2·65 (1·04–6·75) among only the breast-cancer cases that occurred premenopausally (ie, excluding cases who were premenopausal at blood collection but became postmenopausal before the diagnosis of breast cancer). A non-significant inverse association between IGFBP-3 and

	All women			Postmenopausal			Premenopausal			Premenopausal <50 years old		
	Cases/ controls	RR (95% CI)	RR* (95% CI)	Cases/ controls	RR (95% CI)	RR* (95% CI)	Cases/ controls	RR (95% CI)	RR* (95% CI)	Cases/ controls	RR (95% CI)	RR* (95% CI)
Plasma IGF-I quintiles												
<110 ng/mL	92/124	1·0	1·0	81/108	1·0	1·0	..	..	..	..	..	..
110–144 ng/mL	91/119	1·09 (0·74–1·62)	1·10 (0·74–1·65)	82/98	1·22 (0·80–1·86)	1·25 (0·81–1·94)	..	..	..	..	..	..
145–172 ng/mL	66/127	0·77 (0·51–1·16)	0·81 (0·53–1·24)	51/100	0·79 (0·50–1·26)	0·87 (0·53–1·40)	..	..	..	..	..	..
173–219 ng/mL	72/126	0·88 (0·58–1·34)	0·97 (0·62–1·53)	45/85	0·85 (0·53–1·38)	0·95 (0·57–1·61)	..	..	..	..	..	..
≥220 ng/mL	76/124	0·99 (0·65–1·50)	1·06 (0·66–1·70)	46/92	0·85 (0·53–1·39)	0·89 (0·51–1·55)	..	..	..	..	..	..
p for trend		0·86	0·51		0·63	0·99	..	..	..	..	..	..
Plasma IGF-I tertiles												
<158 ng/mL	..	..	..	..	..	..	14/33	1·0	1·0	9/29	1·0	1·0
158–206 ng/mL	..	..	..	..	..	..	27/37	1·63 (0·72–3·72)	1·82 (0·78–4·24)	22/27	2·64 (1·00–6·93)	3·12 (1·14–8·50)
≥207 ng/mL	..	..	..	..	..	..	35/35	2·33 (1·06–5·16)	2·88 (1·21–6·85)	29/22	4·58 (1·75–12·0)	7·28 (2·40–22·0)
p for trend	..	..	..	..	..	..		0·08	0·02		0·02	0·01

RR=relative risk. *Further adjustment for IGFBP-3.

Table 3: **Relative risk of breast cancer by plasma IGF-I quintiles or tertiles**

breast-cancer risk also was found (data not shown).

To define our premenopausal group more stringently, we assessed the same correlations in women who were both premenopausal and younger than 50 years at the time of blood collection. The association between plasma IGF-I concentration and breast-cancer risk increased with a relative risk of 7·28 between the top and bottom tertiles (2·40–22·0) when adjusted for tertiles of IGFBP-3; the addition of other breast-cancer risk factors one at a time to these models did not change the results. When the IGF-I/IGFBP-3 ratio was used, the relative risks (top vs bottom tertiles) in premenopausal and premenopausal women younger than 50 years were 2·13 (0·97–4·68) and 2·46 (0·97–6·24), respectively.

The relative risks comparing the top and the bottom tertile of plasma IGF-I adjusted for IGFBP-3 were similar in the analyses that excluded the 89 women diagnosed in the first year after blood collection. Among premenopausal women (13 cases excluded) and premenopausal women younger than 50 years (ten cases excluded), the relative risks after adjustment for IGFBP-3 were 2·35 (0·94–5·85) and 5·18 (1·66–16·1), respectively.

Discussion

To our knowledge this is the first prospective study to investigate the relation between circulating IGF-I concentrations and risk of breast cancer. We observed a strong positive relation among premenopausal women, particularly after adjusting for concentrations of IGFBP-3, but no association among postmenopausal women.

Two case-control studies[18,21] also found a positive relation between plasma IGF-I concentrations. In the larger study,[18] the relation was strongest among premenopausal women with the ratio of IGF-I to IGFBP-3 most closely related to breast-cancer risk.[18] However, both of these studies were potentially limited by their retrospective design; the blood sample for plasma IGF-I concentration was taken after diagnosis and an effect of the disease on IGF-I concentrations was possible, and the controls may not have been comparable with the cases.[18,21] Our prospective study reduces these concerns to a minimum and suggests that IGF-I concentrations may be a marker of breast-cancer risk among premenopausal women. Although the relation between plasma IGF-I and IGFBP concentrations and IGF-I bioactivity in the tissues is unclear, these epidemiological data suggest they are correlated.

The up to seven-fold increase in breast-cancer risk among premenopausal women 50 years or younger suggests that the relation between IGF-I and risk of breast cancer may be greater than that of other established breast-cancer risk factors—with the exception of a strong family history of breast cancer or a high-density mammographic profile. However, because we had only a small number of premenopausal women, studies that include large numbers of young women are needed to confirm this finding and provide more precise risk estimates. More research is also needed to find out whether known risk factors for breast cancer are acting as surrogates of IGF-I, or, alternatively, whether IGF-I is a marker of other possible risk factors in premenopausal women.

The difference in relative risk between premenopausal and postmenopausal women may reflect an important effect of the growth hormone/IGF-I axis that occurs early in life after breast development. In our study, circulating IGF-I concentrations did not change acutely with menopause but did decrease with age. If this age-related decrease in IGF-I concentrations varies in a random way, with increasing age IGF-I concentrations may become less and less reflective of those in adolescence and early adulthood. Alternatively, IGF-I concentrations may be particularly relevant to the risk of breast cancer in premenopausal women since oestradiol enhances the action of IGF-I in the breast.[22] Although we did not find a relation between postmenopausal IGF-I concentrations and risk of breast cancer, we cannot rule out an effect of premenopausal IGF-I concentrations on the risk of breast cancer after menopause.

There is substantial indirect evidence of a relation between IGF-I and risk of breast cancer. Energy-restricted diets, which decrease IGF-I, decrease the incidence of breast cancer in rats.[23] Also, there is a positive relation between birthweight and breast-cancer risk,[24,25] and low birthweight is associated with a lower IGF-I concentration.[26] Similarly, height, which is positively associated with breast-cancer risk in most epidemiological studies,[27] is positively related to IGF-I concentrations.[11] Furthermore, there is evidence that IGF-I concentrations may be related to risk of prostate and colon cancers.[28,29]

In women who take tamoxifen, circulating IGF-I concentrations decrease[2] and IGF-I bioactivity is reduced because of a decrease in pituitary growth-hormone secretion, IGF-I gene expression and IGF-I-receptor concentrations, and because of upregulation of IGFBP expression.[30] The association between IGF-I concentration and risk of breast cancer provides a novel rationale for the use of tamoxifen as a chemopreventive agent. It will be important to establish whether baseline IGF-I concentrations can predict risk or the potential for risk reduction by antioestrogens and whether IGF-I can be used as an intermediate endpoint in chemoprevention trials. There are several additional agents that lower IGF-I concentrations, such as antagonists of growth-hormone releasing-hormone[31] or growth-hormone,[8] and somato-statin analogues.[32] Some of these are already in clinical breast-cancer trials[33] and may deserve consideration as risk-reducing agents for women who have a high risk related to raised IGF-I concentrations. Interestingly, other proposed agents for breast-cancer prevention, such as retinoids, transforming growth factor-B, and vitamin D analogues, also upregulate IGFBP expression—an action that may be correlated with lower IGF-I bioactivity in certain physiological contexts.[34–36] Finally, the relation between nutritional or other lifestyle factors and concentrations of IGF-I or IGFBP deserves further study.

Contributors
Susan Hankinson coordinated the study, analysed and interpreted the data, and wrote the paper. Walter Willett, Graham Colditz, David Hunter, and Frank Speizer interpreted the data and wrote the paper. Michael Pollak initiated the study of IGF-I, directed the laboratory analysis, interpreted the data, and wrote the paper. Bernard Rosner and Dominique Michaud analysed the data and wrote the paper. Bonnie Deroo did all the assays.

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