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Epidemiology

Interrelationships between serum leptin, IGF-1, IGFBP3, C-peptide and prolactin and breast cancer risk in young women

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Summary

Epidemiologic evidence suggests obese premenopausal women experience a reduced risk of breast cancer. The mechanism underlying this protection is not fully understood although it is well documented that abdominal obesity may impair ovulatory function and reduce gonadal steroidogenesis. We measured levels of several metabolic markers that are modified by obesity [measured by body mass index (BMI, (weight (kg)/height (m^2)))] and play a role in the reproductive axis, including, leptin, insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein 3 (IGFBP3), c-peptide and prolactin in 233 cases and 251 controls participating in a retrospective study of breast cancer in young women conducted in the Seattle/Puget Sound region between 1990 and 1992. Consistent with the finding of a reduced risk with increasing BMI, risks declined with leptin levels, although to a lesser degree with odds ratios (OR) for the highest vs. lowest quartile of BMI = 0.34 (95% C.I. 0.3–0.8) and for leptin = 0.71 (95% C.I. 0.5–1.3). IGF-I, IGFBP3, c-peptide and prolactin were not related to breast cancer risk in a dose-dependent manner. With the possible exception of leptin, our findings do not suggest that these markers explain the breast cancer protection provided by obesity in premenopausal women.

Introduction

The role of obesity in breast cancer etiology is complex. While increasing risk in postmenopausal women [1], most studies in premenopausal women suggest the association with obesity is reversed [2]. The reasons for this reduced risk in menstruating women are not entirely understood although it is well documented that abdominal obesity may impair ovulatory function and reduce gonadal steroidogenesis. We hypothesized that several biomarkers which are modified by obesity and play a role in the reproductive axis may be linked to breast cancer risk in young women.

Leptin, the protein product of the *ob* gene, is involved in the regulation of body weight and composition with serum concentrations determined largely by fat mass. *Ob* gene expression occurs primarily in adipose tissue, but activity has been demonstrated in other tissues, including the breast, where the leptin receptor has also been identified. Leptin has been shown to enhance estrogen concentrations in the breast and to stimulate the proliferation of normal and malignant breast epithelial cells in animal models [3]. Epidemiologic findings are limited. Two investigations have been reported in premenopausal women using bloods collected at the time of case diagnosis [4,5], one of which found reduced breast cancer risk associated with elevated leptin levels [4].

Insulin and IGFs are involved in many functions, including the regulation of energy metabolism [6] and playing a role in the reproductive axis [7]. Included in the IGF family are two polypeptide ligands (IGF-I and IGF-2) and at least six binding proteins, with IGFBP3 the predominant carrier protein for IGF-I. Accumulating evidence suggests insulin induces morphologic alterations in the ductal epithelium of normal, dysplastic, and fibroadenomatous human breast tissue [8], while IGFs help control cell growth, differentiation and apoptosis in normal and neoplastic breast tissues [9]. The link between hyperinsulinemia and breast cancer is not consistent. Most [10–13], but not all case–control studies [14] show an association, and studies using prospectively collected samples do not support this finding [15,16]. Moreover, a reduced breast cancer risk has been suggested for diabetic women in the Nurse's Health Study [17]. IGF-I has been linked to premenopausal breast cancer in some [16,18–20], but not all cohorts [15,21] and

the role of IGFBP3 in breast cancer etiology is inconclusive. Initial reports suggested an inverse relationship with premenopausal disease [19,22], but a recent meta-analysis of data from cohort studies showed modest increased risks in premenopausal women [9].

Prolactin, a peptide hormone secreted by the anterior pituitary, is critical for mammary gland development and differentiation and recent evidence suggests prolactin synthesis and secretion also occurs in the breast, with estrogen enhancing this synthesis. A link between obesity and hyperprolactinemia is well recognized, although the underlying mechanism for this association is not clear. During lactation, prolactin mobilizes lipids from adipose tissue, while in non-lactating women hyperprolactinemia increases appetite and promotes fat mobilization and deposition [23]. The epidemiologic evidence for its role in breast cancer is limited and based on small studies linking prolactin to premenopausal disease [24].

We had the opportunity to investigate the breast cancer risk associated with circulating levels of leptin, non-fasting c-peptide (a marker of endogenous insulin production), IGF-I, IGFBP3, and prolactin in young women who participated in an incident population-based case–control study conducted between 1990 and 1992. Bloods were provided by a subset of participants in the Seattle/Puget Sound component of the study.

Material and methods

Case–control study design and population

The design of this study has been reported [25]. Briefly, subjects were participants in a population-based case–control study of breast cancer in women aged 25–44 conducted between 1990 and 1992 in Atlanta, GA, Seattle/Puget Sound, WA, and five counties in central NJ. Bloods were collected from a subset of participants in the Seattle/Puget Sound region, where incident breast cancer cases diagnosed between May 1990 and December 1992 were identified and frequency matched to controls by 5 year age groups. Controls were identified through random digit dialing using a screening interview to obtain household composition at each number identified in the Seattle/Puget Sound sampling frame. Participation was obtained from 90.5% of the 5722 telephone numbers, and from this, a stratified random sample of controls was selected.

A total of 712 cases and 748 controls were eligible for study, with 644 cases and 610 controls completing the in-person interview (response rates were 90.4% and 81.6%, respectively). The majority of non-respondents were refusals (58 cases, 110 controls), with the remaining due to the subject moving out of the catchment area (7 cases, 19 controls), language problems (1 case, 8 controls) and death (2 cases, 1 control). Overall, the response rate for controls (including participation rates for both the telephone screener and interview) was 73.8%.

Interviews were conducted in-person and obtained information about demographic factors, reproductive and menstrual history including exogenous hormone use, medical history, cancer in family members, alcohol and tobacco use, anthropometry and physical activity. Anthropometric measures were obtained following the interview. Hospital records were abstracted for clinical data and tumor pathology for the cases.

Blood collection and laboratory methods

Initially, attempts to collect bloods were restricted to women with breast cancer who could provide samples prior to surgery and adjuvant therapy and lived within a 45-mile radius of Seattle. Eligibility criteria were later broadened to improve participation rates, and included women who lived outside the proscribed radius and were at least 6 weeks post-surgery but pre-adjuvant therapy. Of the 644 breast cancer cases completing an interview, 405 were not contacted for blood collection, including 326 who were less than 6 weeks post-surgery or undergoing adjuvant therapy, 46 who were surgically or naturally menopausal at the time of interview, 5 who were pregnant or less than 6 months postpartum, 25 who were out of the catchment area, and 3 for other reasons. Of the 239 cases contacted, 233 agreed to donate blood (97.4%). For the controls, blood collection was restricted to a sample of participants in the interview study who lived within the 45-mile radius of the Seattle/Puget Sound region. Of the 610 controls interviewed, 300 were not contacted for the following reasons: 54 were surgically or naturally menopausal at interview; 33 were nursing, pregnant, or less than 6 months postpartum, 208 were out of the catchment area, and 5 were taking hormone supplements or blood thinners. Thus, of the 310 women contacted, 251 (80.9%) donated blood, 52 women refused and 7 did not participate for other reasons.

Bloods were collected using standard procedures, placed on ice, and transported to a repository where serum was aliquotted within 4 h of collection and kept in long-term storage at −70 °C. Assays for leptin, prolactin, c-peptide, IGF-I and IGFBP3 were conducted at Diagnostic Systems Laboratory (DSL), Webster TX. Leptin and prolactin were measured by non-competitive immunoradiometric assays (IRMA) in which the analyte is ''sandwiched'' between two antibodies. C-peptide was measured by radioimmunoassay (RIA) using standard methods, while IGF-I and IGFBP3 were measured by an enzyme-linked immunosorbent assay (ELISA). CVs, obtained by placing four blinded quality control samples randomly into each batch, were as follows: leptin, 5% and 10% (within and between batches, respectively); prolactin, 5% and 9.2% (within and between); c-peptide, 4.4% and 5% (within and between); IGF-I, 5% and 15.8% (within and between); and IGFBP3, 4.5% and 14.3% (within and between). Limits of detection for assays are as follows: leptin, 0.1 ng/ml; IGF-I, 0.03 ng/ml; IGFBP3; prolactin, 0.1 ng/ml; c-peptide, 0.01 ng/ml.

Samples from cases and frequency matched controls were analyzed in the same batch.

Statistical analysis

The initial analyses included all women who participated in the blood collection. To address the concern that levels of circulating hormones may influence some or all of these biomarkers, we also restricted analyses to 168 cases and 195 controls included in an earlier assessment of sex steroid hormones [26]. From interview data obtained at the time of blood collection, this excluded women who were: naturally or surgically menopausal (15 cases, 4 controls), pregnant, <6 months postpartum or <6 months post lactation (5 cases, 8 controls), using oral contraceptives or hormone supplements in past 6 months (20 cases, 32 controls), or other (25 cases, 12 controls). Finally, with the possibility that disease progression may alter concentrations of the biomarkers under study, additional analyses were limited to breast cancer cases diagnosed with stage 0 or 1 disease.

For case–control comparisons, logistic regression models were used to obtain odds ratio estimates (OR) and 95% confidence intervals (CI). Cut points for categories of the metabolic factors were based on the quartile distribution in controls. ORs were adjusted for age (<35, 35–39, 40–44), BMI (quartiles), and time of day of blood collection (morning vs other), and for the subset of women with hormone data, phase of the menstrual cycle (follicular, days 0–11; periovulatory, days 12–16; midluteal, days 17–24; and other). Date of the onset of the last menstrual period was obtained at the time of the blood draw. Adjustment for established breast cancer risk factors including ages at menarche and first birth, alcohol use, race, ethnicity, oral contraceptive use and family history of breast cancer did not alter these findings and were not included in the final models. Tests of trends were obtained by scoring the categories and considering the scored variable as continuous in the logistic regression models. Pearson correlations among metabolic factors in the controls were obtained after logarithmic transformation of the data. Tests of trend for BMI and anthropometric measurements according to breast cancer risk factors in the controls were obtained from standard linear regression models by considering the scored breast cancer risk factor variable as continuous. Analyses were conducted using SYSTAT software [27].

Results

Table 1 presents the distribution of cases and controls by selected demographic characteristics and known breast cancer risk factors including age at menarche, age at first birth, number of births, breast cancer in a first degree relative, and duration of oral contraceptive use. These results are consistent with findings from the full Seattle/Puget Sound study, and with the exception of increased risk for later age at menarche, agree with the breast cancer risk profile for premenopausal women. Cases were slightly older than controls (39 vs 38.2, $p=0.03$), were more likely than controls to have blood drawn early in the day ($p<0.001$), and the majority were diagnosed with cancer *in situ* or at stage 1 [178 cases (77%)]. Although blood collection did not target women who were menopausal at the study interview, an additional 15 cases and 4 controls reported being naturally or surgically menopausal at the time of blood collection. Unless indicated, we included all participants in the blood collection.

ORs for leptin, IGF-1, IGFBP3, c-peptide, prolactin and several anthropometric measures including BMI, height, weight, and waist-to-hip ratio (WHR) are presented in Table 2. Analyses were adjusted for age and BMI (for the markers only) and are shown for the following groups: (a) all women who participated in the blood component of the study (leftmost column); (b) premenopausal women included in the study of circulating sex hormones; and (c) the subset of breast cancer cases diagnosed with stage 0 or 1 disease. Among the entire study group, risks for leptin tended to decline with higher levels, but neither the trend nor the risks were significant (p trend $=0.18$). No consistent pattern of risk was observed for IGF-1, IGFBP3 or c-peptide. ORs decreased with increasing BMI, with the OR for all categories significantly less than one (p trend ≤ 0.05). Risks increased significantly for taller women (p trend $=0.05$) and declined in heavy women, with the OR for the heaviest women being 0.57, $p<0.05$). Risks for WHR showed a similar pattern as BMI.

Restricting analyses to menstruating women, ORs adjusted for phase in menstrual cycle for leptin, the growth factors, c-peptide and prolactin were not significantly different from 1.0 and no trends were observed for leptin, IGF-I or c-peptide. Risks associated with increasing levels of IGFBP3 and to a lesser extent, prolactin, appeared to increase with increasing levels, but trends were not statistically significant. In contrast, ORs associated with the anthropometric indicators varied as observed in the larger study group, with significant trends in risk for all the indicators. Among women diagnosed at stages 0 or 1, risks did not vary in a consistent manner for any of the markers under study and were not significant. ORs for increasing BMI, weight and WHR declined significantly, but risks for height did not increase in a monotonic pattern (p trend $=0.15$).

Metabolic and breast cancer risk factors in controls

Levels of some markers were correlated among controls (Table 3), including leptin with c-peptide ($r=0.34$, $p<0.05$) and prolactin with c-peptide ($r=0.21$, $p<0.05$), but unexpectedly, the correlation between IGF-I and IGFBP3 was low and not significant ($r=0.13$). BMI correlated positively with c-peptide and prolactin, and inversely with IGF-I, but only the coefficient with

Table 1. Case–control distribution of demographic characteristics and odds ratios for selected breast cancer risk factors

	Cases	Controls	Odds ratio*	(95% C.I.)
Age mean, (range)	39 (20–44)	38.2 (25–44)		
Race				
Caucasian	208 (89)	214 (85)		
African American	10 (4)	13 (5)		
Other	15 (6)	24 (10)		
Stage at diagnosis				
0	68 (29)			
1	111 (48)			
2	50 (22)			
3	2			
Education				
High school or less	40 (17)	44 (18)		
Technical school	15 (6)	20 (8)		
Some college	63 (28)	84 (34)		
College graduate	70 (31)	57 (23)		
Post graduate	40 (17)	39 (16)		
Time of blood draw				
Morning	87 (37)	44 (17)		
Afternoon or evening	137 (59)	181 (72)		
Unknown	9 (4)	26 (10)		
Number live births				
None	85 (36)	68 (27)	1.00	
1	43 (18)	45 (18)	0.64	0.27, 1.56
2	71 (30)	84 (33)	0.54	0.24, 1.18
3	26 (11)	28 (11)	0.60	0.26, 1.39
4+	8 (3)	26 (11)	0.23	0.09, 0.59
Age at menarche				
<11	12 (5)	26 (10)	1.00	
11	36 (15)	42 (17)	1.84	0.79, 4.25
12	72 (31)	68 (27)	2.07	0.95, 4.53
13	69 (30)	81 (32)	1.63	0.75, 3.55
14	22 (9)	17 (7)	2.49	0.96, 6.47
15+	22 (9)	16 (6)	2.71	1.02, 7.22
	22 (9)	16 (6)	2.71	1.02, 7.22
Age at first birth				
Never	85 (36)	68 (27)	1.00	
<20	22 (9)	37 (15)	0.48	0.25, 0.91
20–24	37 (16)	47 (19)	0.57	0.33, 1.01
25–29	47 (20)	60 (24)	0.54	0.32, 0.90
30+	42 (18)	39 (15)	0.72	0.41, 1.26
Family history of breast cancer				
Yes	36 (16)	15 (6)	2.79	1.47, 5.32

*Odds ratios are adjusted for age (3 categories).
Except for age, percents are provided in parentheses.

c-peptide was significant ($r = 0.20$; $p = 0.05$). Correlations between weight and the metabolic markers were similar to those observed for BMI, while the coefficients for height and WHR were slight and not significant.

BMI, weight and WHR varied with known breast cancer risk factors among the controls; for descriptive purpose we present box plots of BMI according to categories of each risk factor (Figure 1). BMI declined with later age at menarche ($p < 0.01$) and was significantly different among racial groups ($p = 0.01$), but did not vary consistently according to age at first birth, education, or years of oral contraceptive use. Adult height and weight at blood draw varied significantly by race and age at menarche, while WHR declined with later age at menarche and with increasing years of oral contraceptive use (results not shown). Levels of leptin, c-peptide, IGF-I, IGFBP3 and prolactin did not show consistent patterns according to these breast cancer risk factors.

Table 2. Odds ratios for leptin, growth factors, c-peptide, prolactin and anthropometric indices

	Controls	Cases	Odds ratio[a]	In hormone analysis			Stage 0 or 1 at diagnosis	
				Controls	Cases	Odds ratio[b]	Cases	Odds ratio[a]
	251	233		190	167		176	
Leptin (ng/ml)								
< 16.2	63	72	1.00	49	49	1.00	58	1.00
16.2–27.55	62	59	0.88	49	45	1.14	44	0.79
27.55–45.95	63	56	0.80	45	40	1.12	39	0.69
> 45.95	63	46	0.71	52	35	0.77	38	0.73
p trend			0.18			0.47		0.75
IGF-I (ng/ml)								
< 192.5	63	75	1.00	49	49	1.00	58	1.00
192.5–241.1	62	44	0.52*	48	34	0.60	37	0.58
241.2–300.4	62	56	0.63	48	41	0.67	41	0.61
> 300.4	64	58	0.68	50	45	0.80	43	0.68
p trend			0.77			0.61		0.74
IGFBP3 (ng/ml)								
< 2030.4	58	50	1.00	46	39	1.00	36	1.00
2030.4–2224.1	66	50	0.81	55	40	0.87	37	0.85
2224.2–2659	64	81	1.38	50	53	1.24	61	1.46
> 2659	61	52	1.00	43	37	1.38	45	1.21
p trend			0.50			0.92		0.93
C-peptide (ng/ml)								
< 2.68	62	57	1.00	51	40	1.00	43	1.00
2.68–4.15	62	67	1.26	46	47	1.17	52	1.13
4.16–5.93	63	65	1.19	49	49	1.39	51	1.28
> 5.93	64	43	0.79	49	32	0.86	32	0.80
p trend			0.79			0.80		0.78
Prolactin (ng/ml)								
< 7.60	61	64	1.00	48	45	1.00	51	1.00
7.60–11.19	65	55	0.79	50	41	0.87	43	0.78
11.20–16.68	61	60	0.98	47	42	1.11	46	0.95
≥16.69	64	53	0.86	50	40	1.12	38	0.78
p trend			0.97			0.86		0.79
BMI (kg/m^2)								
< 20	24	14	1.00	9	16	1.00	22	1.00
20–24.9	122	114	0.61	37	54	0.61	53	0.50
25–29.9	62	51	0.46	50	40	0.36*	40	0.36*
30+	55	32	0.34	40	22	0.30*	22	0.27*
p trend			0.003			0.002		0.001
Height (cm)								
< 159.2	46	61	1.00	31	50	1.00	37	1.00
159.2–163.5	52	62	1.11	43	51	1.35	38	1.01
163.6–168.2	59	60	1.31	41	44	1.52	52	1.44
> 168.2	73	63	1.63	53	46	1.93*	49	1.36
p trend			0.048			0.031		0.154
Weight (kg)								
< 59.2	72	60	1.00	53	44	1.00	57	1.00
59.2–66.0	59	61	0.79	45	45	0.83	45	0.76
66.1–78.2	56	62	0.75	41	52	0.65	43	0.72
> 78.2	42	62	0.57*	28	49	0.48*	30	0.53*
p trend			0.048			0.013		0.030

Table 2. (Continued)

	Controls	Cases	Odds ratio[a]	In hormone analysis			Stage 0 or 1 at diagnosis	
				Controls	Cases	Odds ratio[b]	Cases	Odds ratio[a]
Waist to Hip Ratio								
< 0.75	78	62	1.00	56	48	1.00	63	1.00
0.75–0.79	69	61	0.87	58	44	1.07	52	0.80
0.80–0.85	48	62	0.60*	32	50	0.53*	36	0.55*
> 0.85	35	61	0.42*	22	49	0.36*	25	0.37*
p trend			0.001			< 0.001		< 0.00

[a]Odds ratios adjusted for age (< 35, 35–39, 40–44) and for the markers, BMI (4 categories).
[b]Odds ratios adjusted for age (< 35, 35–39, 40–44) and additionally for the markers, BMI (4 categories), time of blood draw (am vs pm) and phase of menstrual cycle (follicular, luteal or other).

Table 3. Correlations among markers in control women

	BMI	Weight	Height	WHR	Leptin	IGF-I	IGFBP3	C-Peptide
BMI	1.00							
Weight	0.99*	1.00						
Height	0.09	0.16	1.00					
WHR	0.11	0.09	−0.11	1.00				
Leptin	0.08	0.07	0.003	0.04	1.00			
IGF-I	−0.13	−0.13	−0.04	−0.17	0.14	1.00		
IGFBP3	−0.05	−0.05	−0.05	−0.08	0.08	0.13	1.00	
C-peptide	0.20*	0.20	−0.04	0.04	0.34*	0.01	0.15	1.00
Prolactin	0.17	0.17	−0.04	−0.04	0.09	−0.03	−0.01	0.21*

Discussion

Consistent with a large number of investigations of breast cancer in premenopausal women, we observed significantly reduced risks for elevated BMI, weight and WHR, while risks for taller women were elevated. Risks associated with increasing levels of serum leptin also declined, although the estimates were not as low as those for BMI and not significant after adjustment for BMI. In contrast, the risk for obese women was significantly reduced, even after adjustment for leptin. No consistent associations were observed between breast cancer risk and levels of IGF-I, IGFBP3, c-peptide or prolactin. Reasons for the reduction in risk of breast cancer associated with obesity in younger women are not known. Most point to the hormonal pathway, since

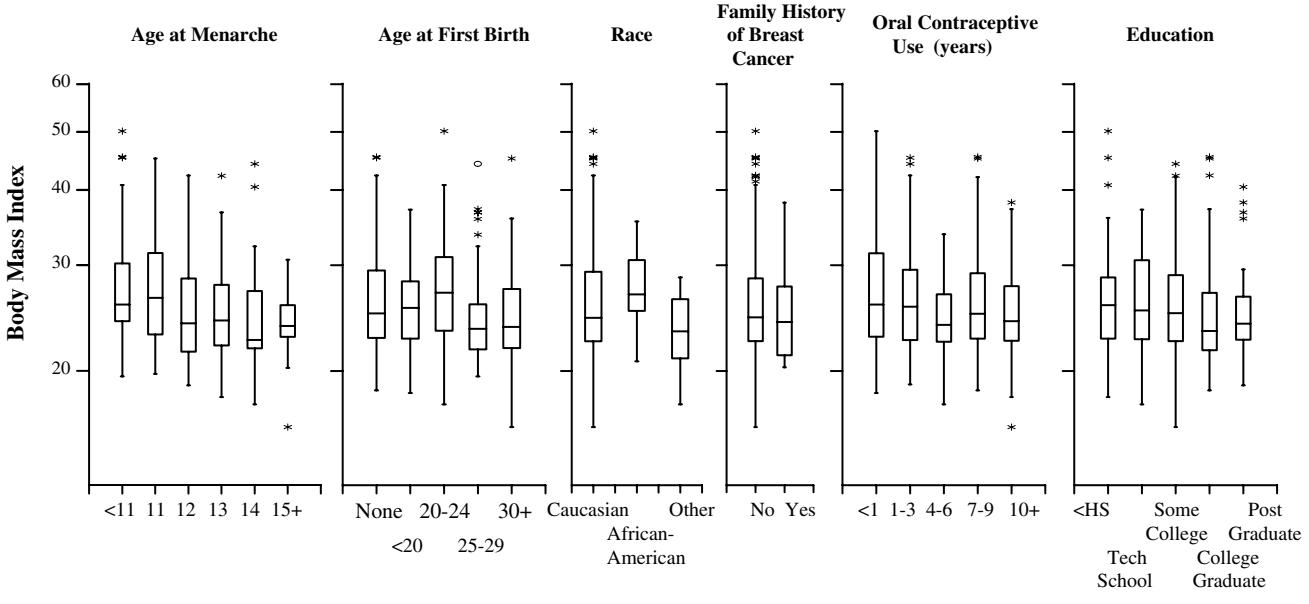

Figure 1. Boxplots of BMI among controls according to breast cancer risk factors.

obesity in premenopausal women is often accompanied by anovulatory or irregular menstrual cycles, conditions associated with altered estrogen profiles [28].

An increasing body of evidence suggests that in addition to its role in energy homeostasis, leptin is involved in the hypothalamic–pituitary–gonadal (HPG) axis and reproduction, although its role in gonadal steroidogenesis is not entirely clear. That sex hormones influence leptin concentrations is supported by several observations, including: leptin concentrations increase at menarche, are higher in women of reproductive age than men of comparable age, and correlate inversely with testosterone levels in males and directly with estradiol in females [29]. In preovulatory and midluteal phase women, leptin concentrations parallel elevations in circulating estradiol and luteinizing hormone (LH) [30] and studies of cycling women show that combined treatment with estradiol and progesterone stimulates leptin secretion [31]. Finally, adipocytes dramatically increase leptin secretion when cultured with estradiol, while androgens inhibit this activity. Evidence is also accumulating that leptin participates in regulating ovarian folliculogenesis, with effects dependent on the hormonal and metabolic environment in the follicle, including levels of IGF-I, insulin and GH [32]. While the results are not entirely consistent, it appears that a minimum level of leptin is required for fertility but that excess levels may limit estradiol secretion in the maturing follicle and hinder ovulation. Our finding of a reduced breast cancer risk associated with elevated leptin in younger women is consistent with this latter action and with findings from another case–control study of leptin in premenopausal disease [4], and supports the argument that the inverse association between BMI and premenopausal breast cancer risk operates through altered hormone profiles. Unfortunately this cannot be adequately addressed at present, since studies of leptin in premenopausal breast cancer have been retrospective in design, where levels of circulating sex hormones are likely to be altered by disease and/or treatment.

Interest in the role of IGFs in breast cancer etiology has been fueled by research demonstrating that these factors may encourage tumor development directly by regulating and enhancing cell growth, differentiation and apoptosis in breast epithelial cells [33–36]. Recent efforts to pool findings from epidemiologic studies that evaluated the role of IGFs in breast cancer [9,37–39] link premenopausal disease and elevated levels of IGF-I and IGFBP3, but findings for both markers are modest when analyses are limited to studies with prospectively obtained samples. While our lack of finding of risk for IGF-I is at odds with the overall conclusion from the pooled studies, similar results have been reported from several studies of premenopausal breast cancer cited in these meta-analyses. This finding was not changed when we excluded women diagnosed with more advanced disease, where IGF-I levels are known to be low.

Several lines of laboratory and clinical evidence, including some case–control studies [10–13] suggest a link between insulin and/or markers of insulin resistance and breast cancer, although results from investigations using samples collected prior to diagnosis do not concur with these findings [15,16]. Our study adds to the preponderance of findings from prospective studies which do not support an association between c-peptide and breast cancer risk.

The role of prolactin in breast cancer is controversial, but the recent discovery of additional forms of the prolactin receptor and the finding that prolactin behaves in an autocrine/paracrine manner in breast tissue [40] has renewed interest in this peptide hormone. Findings from epidemiologic studies are inconclusive, with results based on very small numbers of breast cancer cases. In two of the three cohort studies with findings, prolactin levels were associated with premenopausal breast cancer risk, but not significantly [41–43]. We did not observe an association between serum prolactin and breast cancer risk in this population of younger women.

Finally, we did not observe the established correlation between leptin and BMI among our controls, but as expected from several clinical and experimental studies [30], leptin and c-peptide levels were highly correlated. Levels of IGF-I, IGFBP3, c-peptide and BMI were not well correlated. The relationship between BMI and IGF-I is not well understood, with some suggesting insulin and growth hormone secretion is disrupted in very lean or obese women [44] so that high IGF-I will be found only in women with moderate BMI. While the finding that BMI, weight and WHR declined with later age at menarche is not unanticipated, it may explain the unexpected elevated risk observed for this factor in this study population.

The limitations of our study design warrant discussion, in particular that bloods were collected without regard to time of day, or day within menstrual cycle. Samples were non-fasting, obtained at a single point in time, and for the cases, after breast cancer diagnosis. Although efforts were made to control for the hour of blood draw in the analysis, problems arising from the sample collection may in part explain the null findings. Although c-peptide provides a more reproducible indicator of basal insulin secretion than circulating insulin itself, it is likely our measure in non-fasting samples reflects both recent food intake as well as basal levels. There is little evidence of circadian variability or influence of food intake for the IGFs, but prolactin levels are quite variable since it is secreted episodically, with an early morning peak, and levels are higher during ovulation, nursing, stress and pregnancy. Similarly, leptin is secreted in a pulsatile fashion with an early morning peak. Levels change rapidly after forced feeding or fasting, but normal feeding does not appear to affect concentrations. Finally, it is not likely that null results arose from problems with the assays themselves, since the performance of all the assays was acceptable, with measures of reproducibility comparable to values in other studies in similar populations.

Few efforts had been made to evaluate breast cancer risks associated with leptin, prolactin, c-peptide or other markers of insulin resistance, and growth factors in studies utilizing bloods obtained prior to diagnosis. Findings from case–control studies such as this are inconclusive because levels of circulating biologic markers in subjects for whom samples were collected subsequent to breast cancer diagnosis may be influenced by the disease process and/or treatment protocol. Thus, although we restricted our case–control comparisons to women whose blood was drawn before surgery or at least six weeks post surgery and who were not receiving adjuvant therapy, results must be viewed cautiously. Additionally, imposing these restrictions further lowered our participation rates. With the possible exception of leptin, circulating levels of these metabolic factors do not explain the inverse relationship between premenopausal breast cancer risk and BMI. Future studies of breast cancer in young women using prospectively obtained specimens are needed to explore the interplay between leptin, growth factors, anthropometric indicators and sex steroid hormones.

References

1. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson HE Jr, Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Key TJ, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB, Miller SR, Longcope C: Endogenous hormones breast cancer collaborative group. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst. 95: 1218–1226, 2003
2. Van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, Fraser G, Goldbohm RA, Graham S, Kushi L, Marshall JR, Miller AB, Rohan T, Smith-Warner SA, Speizer FE, Willett WC, Wolk A, Hunter DJ: Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 152: 514–527, 2000
3. Hu Xin, Juneja SC, Maihle NJ, Cleary MP: Leptin-A growth factor in normal and malignant breast cells and for normal mammary gland development. J Natl Cancer Inst 94: 1704–1711, 2002
4. Petridou E, Papadiamantis Y, Markopoulos C, Spanos E, Dessypris N, Trichopoulos D: Leptin and insulin growth factor I in relation to breast cancer (Greece). Cancer Causes Control 11: 383–388, 2000
5. Mantzoros CS, Bolhke K, Moschos S, Cramer DW: Leptin in relation to carcinoma *in situ* of the breast: a study of pre-menopausal cases and controls. Int J Cancer 80: 523–526, 1999
6. Gomez JM, Maravall FJ, Gomez N, Navarro MA, Casamitjana R, Soler J: Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin Endocrinol 58: 213–219, 2003
7. Kaaks R, Bellati C, Venturelli E, Rinaldi S, Secreto G, Biessy C, Pala V, Sieri S, Berrino F: Effects of dietary intervention on IGF-I and IGF-binding proteins, and related alterations in sex steroid metabolism: the Diet and Androgens (DIANA) Randomised. Trial Eur J Clin Nutr 57: 1079–1088, 2003
8. Elias JJ, Armstrong RC: Brief communication: hyperplastic and metaplastic responses of human mammary fibroadenomas and dysplasias in organ culture. J Natl Cancer Inst 51: 1341–1343, 1973
9. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M: Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363: 1346–1353, 2004
10. Bruning PF, Bonfrer JMG, van Noord PAH, Hart AAM, De Jong-Bakker M, Nooijen WJ: Insulin resistance and breast-cancer risk. Int J Cancer 52: 511–516, 1992
11. Del Guidice ME, Fantus IG, Ezzat S, McKeown-Eyssen G, Page D, Goodwin PJ: Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat 47: 111–120, 1998
12. Malin A, Dai Q, Yu H, Shu X, Jin F, Gao Y, Zheng W: Evaluation of the synergistic effect of insulin resistance and insulin-like growth factors on the risk of breast carcinoma. Cancer 100: 694–700, 2004
13. Yang G, Lu G, Jin F, Dai Q, Best R, Shu XO, Chen JR, Pan XY, Shrubsole M, Zheng W: Population-based, case–control study of blood c-peptide levels and breast cancer risk. Cancer Epidemiol Biomarkers Prev 10: 1207–1211, 2001
14. Jernstrom H, Barrett-Connor E: Obesity, weight change, fasting insulin, proinsulin, c-peptide, and insulin-like growth factor-1 levels in women with and without breast cancer: the Rancho Bernardo Study. J Womens Health Gend Based Med 8: 1265–1272, 1999
15. Keinan-Boker L, de Bas Bueno Mesquita H, Kaaks R, van Gils CH, van Noord PAH, Rinaldi S, Riboli E, Seidell JC, Grobbee DE, Peeters PHM: Circulating levels of insulin-like growth factor I, its binding proteins -1, -2, -3, c-peptide and risk of postmenopausal breast cancer. Int J Cancer 106: 90–95, 2003
16. Toniolo P, Bruning PF, Akhmedkhanov A, Bonfrer JMG, Koenig KL, Lukanova A, Shore RE, Zeleniuch-Jacquotte A: Serum insulin-like growth factor-I and breast cancer. Int J Cancer 88: 828–832, 2000
17. Michels KB, Solomon CG, Hu FB, et al Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care 26: 1752–1758, 2003
18. Hankinson SE, Willett WC, Coldtiz GA, Hunter DJ, Michaud DS, Derpp B, Rosner B, Speizer FE, Pollak M: Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 8: 1393–1396, 1999
19. Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, Ram M, Freudenheim JL, Sieri S, Trevisan M, Berrino F: Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 11: 1361–1368, 2002
20. Rinaldi S, Toniolo P, Muti P, Lundin E, Zeleniuch-Jacquotte A, Arslan A, Micheli A, Lenner P, Dossus L, Krogh V, Shore RE, Koenig KL, Riboli E, Stattin P, Berrino F, Hallmans G, Lukanova A, Kaaks R: IGF-I, IGFBP-3 and breast cancer in young women: a pooled re-analysis of three prospective studies. Eur J Cancer Prev 14: 493–496, 2005
21. Kaaks R, Lundin E, Manjer J, Rinaldi S, Biessy C, Soderberg S, Lenner P, Janzon L, Riboli E, Berglund G, Hallmans G: Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in Northern and Southern Sweden. Cancer Causes Control 13: 307–316, 2003
22. Bohlke K, Cramer DW, Trichopoulos D, Mantzoros CS: Insulin-like growth factor-I in relation to premenopausal ductal carcinoma *in situ* of the breast. Epidemiology 9: 570–573, 2003
23. Flint DJ, Binart N, Kopchick J, Kelly P: Effects of growth hormone and prolactin on adipose tissue development and function. Pituitary 6: 97–102, 2003
24. Clevenger CV, Furth PA, Hankinson SE, Schuler LA: The role of prolactin in mammary carcinoma. Endocrine Rev 24: 1–27, 2003
25. Swanson CA, Coates RJ, Schoenberg JB, Malone KE, Gammon MD, Stanford JL, Shorr IJ, Potischman NA, Brinton LA: Body size and breast cancer risk among women under age 45 years. Am J Epidemiol 143: 698–706, 1996
26. Sturgeon SR, Potischman N, Malone KE, Dorgan JF, Daling J, Schairer C, Brinton LA: Serum levels of sex hormones and breast

cancer risk in premenopausal women: a case–control study (USA). Cancer Causes Control 15: 45–53, 2004
27. Wilkinson L: SYSTAT: The System for Statistics. SYSTAT, Inc, Evanston IL, 1986
28. Pasquali R, Pelusi C, Genghini S, Cacciari M, Gambineri A: Obesity and reproductive disorders in women. Hum Reprod Update 9: 359–372, 2003
29. Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A: Leptin in reproduction. Trends Endocrinol Metab 12: 65, 2001
30. Giesthövel F, Meysing A, Brabant G: C-peptide and insulin, but not C^{19}-steroids, support the predictive value of body mass index on leptin in serum of premenopausal women. Human Reprod 13: 547–553, 1998
31. Messinis IE, Papageorgiou I, Milingos S, Asprodini E, Kollios G, Seferiadis K: Oestradiol plus progesterone treatment increases serum leptin concentrations in normal women. Hum Reprod 16: 1827–1832, 2001
32. Brannian JD, Hansen KA: Leptin and ovarian folliculogenesis: implications for ovulation induction and ART outcomes. Semin Reprod Med 20: 103–112, 2002
33. Yu H, Rohan T: Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 92: 1472–1489, 2000
34. Kwandwala HM, et al The effects of insulin-like growth factors on tumorigenesis and neoplasic growth. Endocrin Rev 21: 215–244, 2000
35. Kaaks R, Lukanova A: Energy balance and cancer: the role of insulin and sinlin-like growth factor-I. Proc Nutr Soc 60: 91–106, 2001
36. Thissen JP, Ketelslegers JM, Underwood LE: Nutritional regulation of the insulin-like growth factors. Endocrine Rev 15: 80–101, 1994
37. Sugumar A, Liu YC, Xia Q, Koh YS, Matsuo K: Insulin-like growth factor (IGF)-I and IGF-binding protein 3 and the risk of premenopausal breast cancer: a meta-analysis of literature. Int J Cancer 111: 293–297, 2004
38. Shi R, Yu H, McLarty J, Glass J: IGF-I and breast cancer: a meta-analysis. Int J Cancer 111: 418–423, 2004
39. Fletcher O, Gibson L, Johnson N, Altmann DR, Holly JM, Ashworth A, Peto J, Silva Idos S: Polymorphisms and circulating levels in the insulin-like growth factor system and risk of breast cancer: a systematic review. Cancer Epidemiol Biomarkers Prev 14: 2–19, 2005
40. Vonderhaar BK: Prolactin involvement in breast cancer. Endocr Relat Cancer 6: 389–404, 1999
41. Kwa HG, Cleton F, Wang DY, Bulbrook RD, Bulstrode JC, Hayward JL, Millis RR, Cusick J: A prospective study of plasma prolactin levels and subsequent risk of breast cancer. Int J Cancer 28: 673–676, 1981
42. Hankinson SE, Willett WC, Michaud DS, Manson JE, Colditz GA, Longcope C, Rosner B, Speizer FE: Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 91: 629–634, 1999
43. Wang DY, DeStavola BL, Bulbrook RD, Allen DS, Kwa HG, Fentiman IS, Hayward JL, Millis RR: Relationship of blood prolactin levels and the risk of subsequent breast cancer. Int J Epidemiol 21: 214–221, 1992
44. Allen NE, Appleby PN, Kaaks R, Rinaldi S, Davey GK, Key TJ: Lifestyle determinants of serum insulin-like growth-factor-I (IGF-I), C-peptide and hormone binding protein levels in British women. Cancer Cause Control 14: 65–74, 2003

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