Fasting Glucose Is a Risk Factor For Breast Cancer: A Prospective Study[1]

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Abstract

There is some evidence that glucose and other factors related to glucose metabolism, such as insulin and insulin-like growth-factors (IGFs) may contribute to breast cancer development.

The present study analyzed the hypothesis that serum glucose, insulin levels, and IGF-I pattern are associated with breast cancer using a nested case-control study. Between 1987 and 1992, 10,786 women ages 35–69 were recruited in a prospective study in Italy. Women with history of cancer and on hormone therapy were excluded at baseline. At recruitment, blood samples were collected after a 12-h fast between 7:30 and 9:00 a.m. from all of the study participants.

After 5.5 years, 144 breast cancer cases were identified among the participants of the cohort. Four matched controls were chosen for each breast cancer case from members of the cohort who did not develop breast cancer during the follow-up period.

In premenopausal women, glucose was associated with breast cancer risk: the age, body mass index, and reproductive variable adjusted relative risk (RR) for the highest quartile of serum glucose *versus* the lowest was 2.8 [95% confidence interval (CI), 1.2–6.5], and *P* for trend was 0.02. Insulin showed a weaker association with breast cancer, the adjusted RR of the highest quartile *versus* the lowest was 1.7 (95% CI, 0.7–4.1), and *P* for trend was 0.14, whereas the adjusted RR of the highest quartile of IGF-I was 3.1 (95% CI, 1.1–8.6), and *P* for trend was 0.01. Increased levels of insulin-like growth factor binding protein-3 (IGFBP)-3 were related to breast cancer risk: the adjusted RR for the highest quartile was 2.1 (95% CI, 0.95–4.75), and *P* for trend was 0.02. In postmenopausal women, the associations of glucose, insulin, and IGF-1 pattern were associated with breast cancer risk in heavier subjects characterized by a body mass index higher than 26.

These results indicate that chronic alteration of glucose metabolism is related to breast cancer development.

Introduction

There is biological evidence that glucose and other factors related to glucose metabolism, such as insulin and IGFs[3] may contribute to breast cancer development. Glucose may play a direct role in the development of breast cancer by favoring the "selection" of malignant cell clones (1). Neoplastic cells have been shown to extensively use glucose for proliferation (1). Increased metabolism of glucose toward the pentose phosphate pathways is one of the central metabolic characteristics of malignant tissues (2). In addition, insulin is a powerful mitogenic agent. In cell culture, insulin induces dose-dependent growth response in breast cancer cell lines acting via insulin receptor (3–5). Moreover, insulin may also play a role in tumor promotion by up-regulation of ovarian steroid secretion (6, 7). Intraportal insulin levels influence IGF-I bioavailability (8). IGF-I is a small peptide (~7,500 Da) with significant structural homology with proinsulin and insulin (9), and is highly regulated by growth hormone (10). IGF-I stimulates multiple cellular responses that are related to growth, including synthesis of DNA, RNA, and cellular proteins (11).

There is epidemiological evidence of a close association between major alteration in glucose metabolism and breast cancer risk. In two prospective studies there was a doubling of breast cancer risk for women who had a diagnosis of diabetes at baseline (12, 13). Prospective epidemiological evidence also supports an etiological role of IGF-I in the development of breast cancer (14, 15). Furthermore, consistent with the evidence of a positive association, variables related to insulin resistance such as BMI and abdominal adiposity have been related prospectively to breast cancer risk (16–19).

The purpose of the present prospective nested case-control study was to investigate the association of prospectively mea-

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[3] The abbreviations used are: IGF, insulin-like growth-factor; BMI, body mass index; ORDET, the hormones and diet in the etiology of breast cancer prospective cohort; RR, relative risk; CI, confidence interval; ICC, intraclass correlation coefficient.

sured serum fasting glucose, serum insulin, and IGF-I pattern, defined in the present analysis by IGF-I, free IGF-I, and IGF-I binding proteins IGFBP-1, IGFBP-2, and IGFBP-3, with breast cancer risk. The primary hypothesis of the study was that fasting glucose, insulin, and IGF-I pattern were associated with breast cancer.

Materials and Methods

Between June 1987 and June 1992, 10,786 healthy women, ages 35–69 years, residents of Varese province, Northern Italy, participated in a prospective study of hormones, diet, and breast cancer risk: the ORDET study (20, 21). All members of the cohort were volunteers recruited from the general population through radio, television, and newspaper advertising. Women were also invited to participate in the study through meetings organized by municipalities, local offices of the Italian National Health System, women's associations, churches, and unions. There were 162,700 women between 35 and 69 years of age (the age range of the cohort study) in Varese province during the recruitment phase of the study (22). Thus, the total number of women recruited in the cohort represented ~7% of the general population of women in that age range in Varese province.

The major focus of the ORDET study was the relation of endogenous hormones with breast cancer risk. Thus, at recruitment several sources of hormone variability were controlled for by both inclusion criteria and highly standardized conditions at blood drawing. Women with bilateral ovariectomy, those currently pregnant or breast-feeding, those on oral contraceptives or hormone replacement therapy, or those affected by metabolic diseases influencing the endocrine profile (*i.e.*, liver diseases) were not eligible for the study. In general, 40% of women recruited in the ORDET study had a previous exposure to oral contraceptive, whereas 20% had a previous exposure to hormone replacement therapy. Women with a previous history of cancer were also not eligible. At baseline, information on diet, reproductive history, family history of breast cancer, education, and occupational history were collected together with data on height, weight, and other anthropometric characteristics. On June 1995, after an average of 5.5 years of follow-up, the ORDET data were linked with the local Lombardy Cancer Registry (23, 24) files to identify breast cancer cases and with the regional municipal data of Varese residents to check the vital status of the cohort members. Ten women were considered lost to follow-up, 37 women had been diagnosed with breast cancer before enrollment in the cohort, and 4 were diagnosed with breast cancer *in situ*. Thus, there were 10,735 women available for this study. Among those, 89 died from causes other than breast cancer, and 144 were identified by the cancer registry as cases of invasive breast cancer (73 were premenopausal and 71 postmenopausal at the time of recruitment). Postmenopausal status was defined as the absence of menstrual bleeding for at least 12 months before enrollment.

For each breast cancer case, 4 matched control subjects were randomly chosen from members of the cohort who did not develop breast cancer during the follow-up period. Controls were matched to cases on age (±5 years), menopausal status, daylight saving period at recruitment, recruitment center (there were two recruitment centers), and recruitment period (±89 days).

Among the premenopausal women, there were no stored serum specimens for 4 breast cancer cases and 11 control subjects, and the final analysis included 69 breast cancer cases and 265 control subjects (the 16 control subjects matched to the missing breast cancer cases were also excluded). Among the postmenopausal group of women, 7 breast cancer cases and 18 controls did not have serum samples in the biorepository. Thus, the final analysis included 64 breast cancer cases and 238 control subjects (again, 28 control subjects matched to the missing breast cancer cases were also excluded).

At recruitment, blood samples were collected after 12-h fasting between 7:30 and 9:00 a.m. from all of the participants in the study. For premenopausal women, blood was collected in the luteal phase of the menstrual cycle, between the 20th and 24th day, where the first day of menses was counted as the first day of the ovarian cycle. All of the blood samples were processed and stored at −80°C until biochemical determinations.

Stored serum samples from breast cancer cases and related controls were handled identically and assayed together on the same day and in the same run. All of the laboratory personnel were masked with regard to case-control status. The control of analytical error was based on the inclusion of three standard samples. Serum glucose was determined on a Cobas Mira automated chemistry analyzer (Roche Diagnostic Systems, Indianapolis, IL). The intrabatch coefficient of variation derived from the quality control serum included in the analytical runs was 2.5%. Serum insulin was determined by standard double antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX), with an intrabatch coefficient of variation of 5.2%. IGF-I, free IGF-I, and IGFBPs were determined by double-antibody, immunoradiometric assay (Diagnostic Systems Laboratories, Inc.). The mean intrabatch coefficients of variation derived from the quality control serum included in the analytical runs were 4.5% for IGF-I, 12.8% for free IGF-I, 2.8% for IGFBP-1, 6.4% for IGFBP-2, and 4.3% for IGFBP-3.

Intraindividual variability for fasting serum glucose, insulin, IGF-I, free IGF-I, and the IGF-I binding proteins was evaluated in a sample of 59 cohort members who were asked to come to the recruitment center for a second visit 1 year after enrollment (25). Exclusion criteria applied at the second drawing were as follows: pregnancy or breastfeeding, diagnosis of cancer, and change in menopausal status between the first and the second visit. Both first and second blood drawing were performed 1 year apart in the same month, on the same day of the month, and at the same hour and min of the day between 7:30 and 9:00 a.m. after an overnight fast. In premenopausal women, the two blood draws were taken on the same day of the luteal phase of the menstrual cycle.

Statistical Analysis. Means and SDs for serum glucose, insulin, IGF-I, free IGF-I, IGFBP-1, IGFBP-2, IGFBP-3 and for other risk factors for breast cancer were computed, and compared for cases and control subjects with one-way ANOVA. Means of the variables were also computed for premenopausal and postmenopausal women and compared with the *t* test for unpaired samples. Because of skewed distributions, serum glucose, insulin, IGFBP-1, IGFBP-2, and free IGF-I were log transformed for all of the analyses. Quartiles of exposure for the relevant variables were calculated based on the distribution of the controls. We estimated RRs (26) and 95% CIs using conditional logistic regression. For adjusted analyses, we controlled for age, BMI, waist-to-hip ratio, and social and economic status, expressed as category of employment, and reproductive variables including age at menarche, age at first child, parity, and age at menopause. Models also were fit using the continuous data to test for linear trends.

Interactions between each component of glucose metabolism and IGF-I pattern with menopausal status were tested in the logistic regression models by examination of the product

Table 1 Baseline characteristics of breast cancer cases and controls by menopausal status

	Premenopausal women		Postmenopausal women	
	Cases (n = 69) mean (±SD)	Controls (n = 265) mean (±SD)	Cases (n = 64) mean (±SD)	Controls (n = 238) mean (±SD)
Age (yrs)	44.8 (5.0)	44.4 (4.8)	58.1 (5.5)	57.6 (5.3)
BMI (kg/m^2)	24.3 (3.9)	24.6 (4.6)	26.0 (4.0)	26.7 (4.2)
Waist-to-hip ratio	0.78 (0.075)	0.77 (0.06)	0.81 (0.06)	0.82 (0.08)
Age at menarche (yrs)	12.7 (1.5)	12.7 (1.5)	13.2 (1.6)	13.3 (1.6)
Age at menopause (yrs)	—	—	49.0 (5.2)	48.6 (5.2)
Age at first child (yrs)	25.8 (4.5)	26.0 (4.4)	26.5 (5.1)	26.3 (4.6)
Number of children	1.8 (1.1)	1.9 (1.1)	1.8 (1.0)	2.1 (1.6)[a]
Glucose (mg/dl)	81.9 (18.9)	78.6 (10.8)[a]	82.2 (23.2)	83.9 (34.0)
Insulin (m IU/l)	9.8 (5.9)	9.1 (4.4)	10.5 (7.2)	10.9 (7.6)
IGF-I (ng/ml)	170.1 (55.2)	158.8 (59.8)	123.9 (44.3)	130.1 (50.0)
Free IGF-I (ng/ml)	1.20 (0.7)	1.16 (0.8)	1.26 (0.7)	1.41 (0.5)
IGFBP-1 (ng/ml)	31.9 (21.4)	30.1 (18.3)	38.9 (18.6)	37.8 (21.02)
IGFBP-2 (ng/ml)	415.6 (189.2)	427.6 (342.9)	436.2 (251.1)	410.1 (193.6)
IGFBP-3 (ng/ml)	3754.1 (965.1)	3549.2 (753.4)[a]	3690.0 (1025.6)	3739.8 (806.0)
Employment (prevalence)				
1 low social class	66.7	69.1	68.8	80.7
2 middle class	20.3	12.4	15.6	6.7
3 high middle class	13.0	17.0	15.6	11.3
4 high social class	0.00	1.5	0.00	1.3

ıe-way ANOVA ($P < 0.05$): differences between cases and controls.

terms for each considered variable and menopausal status. Similarly, interactions between each component of glucose metabolism and IGF-I pattern with BMI and with waist-to-hip ratio were examined.

Reliability of hormone determinations was evaluated by the ICC (27).

Results

The ICCs and the lower limit of the 95% CI, reported in parentheses, were: 0.72 (0.52), 0.70 (0.49), 0.81 (0.68), and 0.79 (0.65) for glucose, insulin, IGF-I, and free IGF-I, respectively. For IGFBPs, ICCs were 0.89 (0.82), 0.82 (0.69), and 0.60 (0.33) for IGFBP-1, IGFBP-2, and IGFBP-3, respectively. There were no systematic differences in reliability across all of the considered biomarkers by menopausal status or age groups (less than or older than 49 years, which was the median age of women in this reliability study). IGF-I and glucose measurements were the only two variables characterized by a particularly larger error in premenopausal than in postmenopausal women. ICCs were 0.59 (0.15) and 0.53 (0.15) *versus* 0.85 (0.69) and 0.74 (0.45). On the contrary, insulin was characterized by a notably larger variability in postmenopausal women than in premenopausal women. ICCs were 0.81 (0.65) *versus* 0.67 (0.44–0.87) in premenopausal and in postmenopausal women, respectively.

In Table 1, we report descriptive data on the study participants. Serum glucose and insulin levels were ~5% and 15% lower for premenopausal than for postmenopausal women. The premenopausal women also had 30% higher IGF-I levels, and 15% lower free IGF-I, 20% lower IGFBP-1, and 5% lower IGFBP-3. Premenopausal women were thinner, younger at menarche, younger at their first birth, and had less abdominal adiposity. We tested interaction terms of each of the IGF pattern and glucose metabolism variables with menopausal status, and interactions were not significant for insulin, free IGF-I, IGFBP-1, and IGFBP-2; they were borderline statistically significant for IGF-I and IGFBP-3 (P = 0.1), and statistically significant for glucose (P = 0.04). The evidence of those statistically significant interactions between IGF-I, IGFBP-3, and glucose with menopausal status, and the general evidence that the effect of reproductive factors and BMI differ according to menopausal status (28–31), led us to conduct separate analyses in premenopausal and postmenopausal women.

In premenopausal women, serum fasting glucose was correlated positively with insulin, BMI, and waist-to-hip ratio (r = 0.29, r = 0.20, and r = 0.15, respectively, with $P < 0.01$). It was also negatively correlated with IGFBP-1 (r = -0.20; $P < 0.001$). In postmenopausal women, fasting glucose was only related to insulin (r = 0.37; $P < 0.0.001$) and with IGFBP-1 (r = 0.31; $P < 0.001$). In both premenopausal and postmenopausal women, insulin was positively related to BMI (r = 0.30 for both groups; $P < 0.005$) and waist-to-hip ratio (r = 0.20 for both groups; $P < 0.005$). In addition, it was negatively correlated with IGFBP-1 ($r = -0.29$; $P < 0.001$) in premenopausal women and with IGFBP-2 (r = 0.2 $P < 0.005$) in postmenopausal women.

IGF-I was correlated with IGFBP-3 (r = 0.42 and r = 0.52 in premenopausal and in postmenopausal women, respectively; $P < 0.001$) and negatively with age, IGFBP-1 and IGFBP-2 ($r = -0.26$, $r = -0.28$, $r = -20$, respectively in premenopausal and $r = -0.24$, $r = -0.39$, $r = -35$, in postmenopausal women; $P < 0.005$).

BMI and waist-to-hip ratio were correlated with an r = 0.28 ($P < 0.001$) in premenopausal and r = 0.31 ($P < 0.001$) in postmenopausal women. Breast cancer risks in relation to quartiles of glucose, insulin, IGF-I, free IGF-I, and IGF-I binding proteins are shown in Table 2 and Table 3 for premenopausal and postmenopausal women.

Premenopausal women in the highest quartile of fasting glucose were at almost three times higher risk of developing incident breast cancer compared with those in the lowest quartile (adjusted RR, 2.76; 95% CI, 1.18–6.46; P for trend = 0.04). In the same group of women, insulin showed a slightly higher risk in the third and fourth quartiles. All of the CIs included unity, and there was no evidence of a linear dose-effect relation (P for trend = 0.32). Breast cancer risk increased

Table 2 Estimated risks (RR) of premenopausal breast cancer and 95% confidence limits by serum levels of fasting glucose, insulin, IGF-I, and IGFBPs

	I[a]	II	III	IV	*P* for trend
Glucose					
Crude RR (95% CI)	1.00	1.11 (0.50–2.46)	1.13 (0.49–2.58)	2.41 (1.06–5.48)	0.04
Adjusted RR (95% CI)[b]	1.00	1.09 (0.48–2.44)	1.21 (0.51–2.85)	2.76 (1.18–6.46)	0.02
No. case/control	15/73	14/63	14/66	26/63	
Quartile cut-off (mg/dl)	≤71	>71–≤78	>78–≤84	>84	
Insulin					
Crude RR (95% CI)	1.00	1.12 (0.49–2.55)	1.58 (0.75–3.30)	1.32 (0.60–2.90)	0.32
Adjusted RR (95% CI)[b]	1.00	1.22 (0.52–2.87)	1.78 (0.81–3.90)	1.72 (0.71–4.15)	0.14
No. case/control	16/76	13/57	22/66	18/66	
Quartile cut-off (mIU/ml)	≤5.49	>5.49–≤7.60	>7.60–≤10.96	>10.96	
IGF-I					
Crude RR (95% CI)	1.00	2.30 (0.92–5.74)	3.22 (1.30–7.98)	2.75 (1.04–7.28)	0.03
Adjusted RR (95% CI)[b]	1.00	2.3 (0.91–5.83)	3.66 (1.45–9.27)	3.12 (1.13–8.60)	0.01
No. case/control	8/67	18/66	24/66	19/66	
Quartile cut-off (ng/ml)	≤115.17	>115.17–≤149.36	>149.36–≤199.10	>199.10	
Free IGF-I					
Crude RR (95% CI)	1.00	0.80 (0.34–1.85)	1.65 (0.78–3.67)	1.40 (0.58–3.36)	0.22
Adjusted RR (95% CI)[b]	1.00	0.86 (0.37–7.01)	1.77 (0.82–3.82)	1.55 (0.63–3.80)	0.16
No. case/control	16/71	11/62	23/66	19/66	
Quartile cut-off (ng/ml)	≤0.64	>0.64–≤0.90	>0.90–≤1.52	>1.52	
IGFBP-1					
Crude RR (95% CI)	1.00	1.13 (0.54–2.33)	0.83 (0.36–1.87)	1.10 (0.50–7.42)	0.97
Adjusted RR (95% CI)[b]	1.00	1.11 (0.52–2.37)	0.74 (0.30–1.77)	0.96 (0.39–2.38)	0.76
No. case/control	17/67	19/66	14/66	19/66	
Quartile cut-off (ng/ml)	≤16.59	>16.59–≤24.48	>24.48–≤40.93	>40.93	
IGFBP-2					
Crude RR (95% CI)	1.00	0.72 (0.34–1.53)	0.96 (0.46–1.98)	0.79 (0.34–1.82)	0.73
Adjusted RR (95% CI)[b]	1.00	0.67 (0.30–1.46)	0.85 (0.38–1.89)	0.66 (0.26–1.64)	0.48
No. case/control	20/67	14/66	19/66	16/66	
Quartile cut-off (ng/ml)	≤282.03	>282.03–≤381.990	>381.990–≤519.18	>519.18	
IGFBP-3					
Crude RR (95% CI)	1.00	1.06 (0.44–2.56)	2.09 (0.91–4.79)	2.30 (0.98–5.41)	0.02
Adjusted RR (95% CI)[b]	1.00	1.05 (0.43–2.56)	2.12 (0.91–4.95)	2.31 (0.97–5.53)	0.02
No. case/control	12/67	12/66	22/67	23/65	
Quartile cut-off (ng/ml)	≤3091	>3091–≤3482	>3482–≤3986	>3986	

[a] I, Reference category.
[b] Adjusted for age, BMI, social and economic status, and reproductive variables.

with increasing serum IGF-I, the upper quartile adjusted RR was 3.12 (95% CI, 1.13–8.60; *P* for trend = 0.03). No significant association between free IGF-I and IGFBP-1, and breast cancer was found in premenopausal women. However, there was some suggestion of an increased risk associated with free IGF-I levels above the median (adjusted RR = 1.80; 95% CI, 0.99–3.36 for levels above *versus* below the median). Finally, in premenopausal women, higher levels of IGFBP-3 (highest quartile *versus* lowest quartile) were linked to higher risk of breast cancer (adjusted RR = 2.3; 95% CI, 0.97–5.53; *P* for trend = 0.02).

In premenopausal women, we performed an additional analysis by strata of age at diagnosis: the analysis was done in premenopausal women who had breast cancer diagnosed before (36 breast cancer cases and 138 control subjects) and after 48 years of age (33 breast cancer cases and 127 control subjects), the median age at menopause in controls. In general, the associations of fasting glucose, insulin and IGF-1 pattern with breast cancer were stronger in women recruited in premenopausal status who were diagnosed with breast cancer after 48 years age. However, the point estimates were generally characterized by very large CIs. The relation was particularly stronger in women who had breast cancer diagnosed in an older age for IGF-1, and free IGF-1 for which age at diagnosis displayed a statistically significant multiplicative interaction when the interaction was tested in the logistic regression model ($P < 0.03$, and $P < 0.02$, respectively). The BMI and age adjusted RRs for IGF-1 in the strata characterized by age at diagnosis <48 was 1.51 (95% CI, 0.53–4.29) for the second tertile, and 1.52 (95% CI, 0.50–4.60) for the third tertile, whereas the RRs for IGF-1 in women diagnosed with breast cancer when they were >48 years of age were 2.46 (95% CI, 0.65–9.29) and 15.43 (95% CI, 3.25–73-19) for the second and third tertiles, respectively. For free IGF-1, the risks were 0.55 (95% CI, 0.17–1.71) and 0.74 (95% CI, 0.23–2.34) for the second and third tertiles in women diagnosed before age 48, and 2.24 (95% CI, 0.63–7.92) and 4.97 (95% CI, 1.33–18.54) for the second and third tertiles, respectively, in women diagnosed after age 48.

Associations of breast cancer risk with glucose, insulin, and IGF-I pattern for postmenopausal women were generally weaker than for premenopausal women and not statistically significant.

Results for premenopausal and postmenopausal women were similar to those shown in Tables 2 and 3 when waist-to-hip ratio was added as a confounder. Adjusting RRs for glucose, insulin, IGF-I, and free IGF-I for IGFBPs also did not alter estimates appreciably. Furthermore, the adjustment of insulin for glucose, glucose for insulin or IGF-I, and IGF-I for insulin or glucose did not alter the point estimates shown in Table 2 and in Table 3 as well.

Twenty-one women (4 women who later developed breast

Table 3 Estimated risks (RR) of postmenopausal breast cancer and 95% confidence limits by serum levels of fasting glucose, insulin, IGF-I, and IGFBPs

	I[a]	II	III	IV	*P* for trend
Glucose					
Crude RR (95% CI)	1.00	1.77 (0.79–3.95)	2.23 (0.93–5.37)	1.27 (0.49–3.26)	0.52
Adjusted RR (95% CI)[b]	1.00	1.92 (0.83–4.42)	2.65 (1.06–6.66)	1.63 (0.59–4.46)	0.23
No. case/control	11/61	21/66	20/55	12/56	
Quartile cut-off (ng/ml)	≤71	>71–≤79	>79–≤86	>86	
Insulin					
Crude RR (95% CI)	1.00	0.56 (0.24–1.30)	0.58 (0.25–1.33)	0.69 (0.31–1.51)	0.42
Adjusted RR (95% CI)[b]	1.00	0.56 (0.23–1.37)	0.59 (0.25–1.40)	0.85 (0.36–2.00)	0.76
No. case/control	21/60	13/59	14/60	16/59	
Quartile cut-off (mIU/l)	≤6.09	>6.09–≤8.62	>8.62–≤12.53	>12.53	
IGF-I					
Crude RR (95% CI)	1.00	0.93 (0.43–2.00)	0.95 (0.45–2.01)	0.56 (0.24–1.78)	0.21
Adjusted RR (95% CI)[b]	1.00	1.04 (0.46–2.33)	1.04 (0.48–2.25)	0.58 (0.24–1.36)	0.25
No. case/control	19/60	17/59	18/60	10/59	
Quartile cut-off (ng/ml)	≤93.36	>93.36–≤127.53	>122.53–≤161.14	>161.14	
Free IGF-I					
Crude RR (95% CI)	1.00	0.66 (0.28–1.55)	0.71 (0.29–1.74)	1.41 (0.61–3.23)	0.28
Adjusted RR (95% CI)[b]	1.00	0.61 (0.25–1.51)	0.68 (0.27–1.70)	1.39 (0.59–3.28)	0.30
No. case/control	17/58	12/64	11/55	24/61	
Quartile cut-off (ng/ml)	≤0.79	>0.79–≤1.03	>1.03–≤1.39	>1.39	
IGFBP-1					
Crude RR (95% CI)	1.00	1.63 (0.73–3.62)	0.95 (0.40–2.26)	1.17 (0.81–3.87)	0.33
Adjusted RR (95% CI)[b]	1.00	1.54 (0.66–3.62)	0.85 (0.34–2.13)	1.70 (0.70–4.15)	0.50
No. case/control	12/60	19/59	11/60	22/59	
Quartile cut-off (ng/ml)	≤22.99	>22.99–≤34.89	>34.89–≤46.23	>46.23	
IGFBP-2					
Crude RR (95% CI)	1.00	0.85 (0.39–1.85)	0.20 (0.06–0.64)	1.07 (0.52–2.21)	0.85
Adjusted RR (95% CI)[b]	1.00	0.92 (0.40–2.09)	0.19 (0.06–0.63)	0.87 (0.39–1.92)	0.36
No. case/control	20/60	16/59	5/60	23/59	
Quartile cut-off (ng/ml)	≤264.69	>264.69–≤394.995	>394.995–≤517.04	>517.04	
IGFBP-3					
Crude RR (95% CI)	1.00	0.82 (0.39–1.73)	0.89 (0.62–1.86)	0.69 (0.30–1.60)	0.46
Adjusted RR (95% CI)[b]	1.00	0.78 (0.35–1.71)	0.85 (0.39–1.84)	0.73 (0.30–1.74)	0.53
No. case/control	17/58	12/64	11/55	24/61	
Quartile cut-off (ng/ml)	≤3167	>3167–≤3676.5	>3676.5–≤4266	>4266	

[a] Reference category.
[b] Adjusted for age, BMI, social and economic status, and reproductive variables.

cancer and 17 control subjects) were diagnosed with diabetes (either type 1 or type 2) before the enrollment in the study. In addition, 12 women (3 breast cancer and 9 control subjects) showed serum fasting glucose levels at baseline that were >126 mg/dl, the threshold value for the definition of clinical diabetes (32). We repeated all of the analyses with the exclusion of these 33 subjects and the controls matched to the 7 breast cancer cases (28 subjects), and found that the point estimates were similar to those based on the entire group of women.

Because we had observed previously that BMI modified the effect of abdominal adiposity, as a marker of insulin resistance, on breast cancer risk (16), we performed an analysis of breast cancer risk within strata of BMI and waist-to-hip ratio. Strata were determined by the median for controls. Matching was retained and only the case-control sets that matched on BMI stratum were included. We also conducted an unmatched analysis that included all of the pairs, but the results did not differ and, therefore, only the results for the matched analysis are presented here. In premenopausal women, point estimates were similar in the strata defined by low and high BMI. The age and reproductive variable adjusted RR for the highest tertile of glucose was 2.21 (95% CI, 0.80–5.50) in the stratum at low BMI (BMI ≤24) *versus* 1.57 (95% CI, 0.57–4.36) in the stratum at high BMI (BMI >24). The adjusted RRs for low and high BMI were 1.03 (95% CI, 0.35–3.09) *versus* 1.22 (95% CI, 0.39–3.80) for insulin, 2.04 (95% CI, 0.71–5.85) *versus* 2.40 (95% CI, 0.86–6.66) for IGF-1, and 1.09 (95% CI, 0.41–2.84) *versus* 1.98 (95% CI, 0.65–6.02) for IGFBP-3. We observed similar results for free IGF-1, IGFBP-1, and IGFBP-2 across BMI strata. In postmenopausal women there was a suggestion of effect modification by BMI (Table 4). The data need to be interpreted cautiously because of the low sample size in each cell. The point estimates for glucose, insulin, IGF-I, and free IGF-I appeared to be higher in women with higher BMI. When multiplicative interaction was examined in the logistic regression model, we observed significant interactions of BMI with insulin, free IGF-I, IGFBP-1, and IGFBP-2. For both premenopausal and postmenopausal women in the analysis stratified by waist-to-hip ratio, there was no evidence of effect modification by abdominal adiposity (data not shown).

Discussion

The most interesting findings of the present study is the association of fasting glucose with breast cancer risk in premenopausal women and in heavier postmenopausal women. We found previously in this population that abdominal adiposity was related to breast cancer risk only in premenopausal women (16). Schoen *et al.* (33) reported that fasting glucose was associated with colorectal cancer, another type of cancer of which the etiology has been related to impaired fasting glucose and hyperinsulinemic insulin resistance. Another prospective

Table 4 Estimated risk of postmenopausal breast cancer associated with glucose metabolism and IGF-I factors by strata of BMI[a]

Tertiles of Variables	LOW BMI ≤26			HIGH BMI >26			
	I	II	III	I	II	III	P^b
Glucose							
RR (95% CI)	1.00	1.90 (0.80–4.54)	0.86 (0.26–2.80)	1.00	2.24 (0.70–7.16)	2.05 (0.62–6.76)	0.61
No. case/control	12/48	18/41	5/27	5/36	13/42	11/44	
Insulin							
RR (95% CI)	1.00	0.36 (0.13–0.98)	0.65 (0.23–1.79)	1.00	1.20 (0.30–4.73)	2.16 (0.62–7.52)	0.06
No. case/control	21/48	7/41	7/27	4/27	9/46	16/49	
IGF-I							
RR (95% CI)	1.00	0.68 (0.26–1.78)	0.55 (0.20–1.48)	1.00	1.18 (0.43–3.27)	1.31 (0.45–3.80)	0.14
No. case/control	14/35	11/37	10/40	9/42	11/44	9/36	
Free IGF-I							
RR (95% CI)	1.00	0.40 (0.14–1.16)	0.58 (0.20–1.68)	1.00	2.40 (0.69–8.28)	5.5 (1.61–18.71)	0.003
No. case/control	14/31	9/42	12/43	5/52	10/40	14/30	
IGFBP-1							
RR (95% CI)	1.00	0.73 (0.24–2.25)	1.36 (0.49–3.78)	1.00	0.62 (0.23–1.67)	0.59 (0.18–1.88)	0.03
No. case/control	7/25	9/43	19/48	15/53	9/42	5/27	
IGFBP-2							
RR (95% CI)	1.00	1.14 (0.39–3.31)	1.40 (0.52–3.74)	1.00	0.68 (0.26–1.76)	0.58 (0.19–1.80)	0.02
No. case/control	9/36	9/35	17/45	13/43	10/47	6/32	
IGFBP-3							
RR (95% CI)	1.00	0.84 (0.32–2.18)	0.88 (0.33–2.36)	1.00	1.05 (0.38–2.93)	0.78 (0.26–2.29)	0.16
No. case/control	12/36	12/39	11/41	10/42	11/41	8/39	

[a] Point estimates were adjusted for age and reproductive variables.
[b] *Ps* for interactive terms of each variable with BMI.

study, the Malmö Diet and Cancer Study, did not find an association between fasting glucose and breast cancer risk in premenopausal or postmenopausal women (34). However, in that study the definition of postmenopause allowed for potential misclassification of menopausal status. For example, women in that study were classified as postmenopausal if they stated that their menstruations had ceased without indication for how long they missed periods, if they self-reported symptoms of postmenopause or if they were taking any "female hormonal medication" because of such symptoms. Because menopausal status appears to be a key variable in our study, misclassification of menopausal status could have affected the association between fasting glucose and breast cancer risk in the Malmö study.

In our study, the association of serum fasting glucose appeared to be independent of the levels of insulin and IGF-I because adjustment for those variables did not substantially modify the risk estimates. Fasting glucose levels, after an overnight fast, depends on the hepatic and renal gluconeogenesis (35). Apart from reduction in insulin sensitivity or insulin secretion, which cause increased glucose production and decrease glucose utilization (36), gluconeogenesis is stimulated by counter-regulatory hormones such as adrenal hormones, epinephrine, and cortisol, and by androgens and growth hormones (37, 38). These hormones are determinants of morning fasting glucose, and additional studies are needed to clarify the potential etiological role of these hormones in breast cancer. On the other hand, one can speculate that increased serum glucose availability may offer a selective advantage to malignant cells with increased serum glucose requirement (1). In addition, glucose itself may support carcinogenic processes through the generation of free radicals, and the induction of oxidative damage to both DNA and to the enzymes involved in the repair and processing of DNA (39–43).

In our study, there was a modest association of insulin levels with breast cancer risk, particularly in premenopausal women and in overweight postmenopausal women. Insulin has recently obtained attention as metabolic factor related to risk of breast cancer and colon cancer (44–46). Insulin has a mitogenic effect on mammary epithelium cells (3–5), and it has been observed that the insulin receptor is overexpressed in both human breast cancer and human breast tissues (47–50).

There is now accumulating evidence linking IGF-I to several types of cancers (51). In particular for breast cancer, it is possible that variables related to glucose metabolism and insulin resistance may be of etiological relevance only in younger women. This hypothesis is supported by the consistency of the association between prediagnostic IGF-I and breast cancer only in premenopausal women (Refs. 14, 15 and our present results). In our study, total IGF-I was more strongly associated with breast cancer risk than free IGF-I. We found a suggestion of a potential threshold effect: risk increased for women with free IGF-I levels above the median level. There is evidence that the binding proteins determine bioavailability of IGF-I and that only the fraction of IGF-I bound to IGFBPs is protected against rapid degradation (10, 52). Thus, free IGF-I may exert a permissive effect toward cancer development only if its levels are high enough to exceed the degradation process.

IGFBP-2 has shown to have a protective effect in our and other studies (51). The role of IGFBP-3 in the regulation of breast cancer cell growth is however unclear: both growth inhibition and stimulation have been documented in tissue culture systems and epidemiological investigations (51, 53). One of the proposed functions of IGFBPs is to increase the half-life of IGFs in circulation (10, 52). Thus, IGFBP-3 may enhance the action of IGF-I by protecting it from degradation (54). Another role of IGFBPs is to regulate IGF action by modulating IGF-I bioavailability at the target tissue and IGF-I binding to the receptor (55). We speculate that a key role in development of breast cancer is not only linked to the absolute IGFBPs levels, but rather to an imbalance in IGF-I and binding proteins concentration leading to a perturbation of the IGF milieu.

Finally, we found effect modification by menopausal status for the association between the variables related to glucose

metabolism and IGF-I pattern. It is possible that the effect of menopausal status on the association observed in the present study is explained in part by differences in the estrogen milieu. The principal endocrine change of menopause is a decrease in estrogen serum levels (56). There is evidence that estrogens increase the levels of cellular IGF-I and that IGF-I up-regulates responses to estrogen at the receptor level (57). However, we also found that most of the factors related to glucose metabolism and IGF-I pattern were associated with an increased risk of breast cancer among heavier postmenopausal women. Thus, the effect of BMI on the considered association with breast cancer may be explained by both the relation between increased body fat with insulin resistance and secretion of IGF-I (58), and by the increased availability of estrogens because of the aromatization of androgens in adipose tissue.

Limitations of this investigation warrant consideration. The results of this study are based on a relatively few breast cancer cases, and estimates may therefore be imprecise. Intra-individual variability and the long-term effect of cryopreservation are additional factors potentially affecting our serum measures (59, 60). However, we have found that the variables included in the study are generally characterized by good reliability, and cases and controls were matched on date of sample collection. In addition, the present findings could reflect alteration in glucose metabolism and IGF-I pattern as indicators of undiagnosed breast cancer rather than a cause-effect association. To investigate this hypothesis, we repeated the analysis within 51 premenopausal women who developed breast cancer at least 12 months after their recruitment to the study. Risk estimates were similar to those presented here.

In conclusion, this study shows that fasting glucose is a predictor of breast cancer. In addition, we observed a strong relation of IGF-I and IGFBP-3 with breast cancer risk and moderate association with insulin. The associations were strong in premenopausal women, particularly in those who had breast cancer diagnosed after 48 years of age, that is potentially during the postmenopausal period, and in heavier women already in postmenopausal status at recruitment. Additional studies are needed to clarify the exact role of glucose metabolism pathways in breast cancer development and their differential effect in premenopausal *versus* postmenopausal women.

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