Serum Fructosamine and Subsequent Breast Cancer Risk: A Nested Case-Control Study in the ORDET Prospective Cohort Study

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Abstract

There is evidence that abnormal glucose metabolism may contribute to the risk of breast cancer. The measurement of markers of glucose metabolism could help to identify women at risk for breast cancer. Serum fructosamine is one such marker. In this study, we investigated whether prediagnostic serum fructosamine was associated with breast cancer. Between 1987 and 1992, 10,786 women ages 35 to 69 were recruited in Italy for a prospective study. Women with a history of cancer or on hormone therapy were excluded at baseline. Blood samples were collected after 12 hours fasting from all participants at recruitment. After 5.5 years of follow-up, 144 breast cancer cases were identified and four matched controls were selected from the cohort; serum fructosamine levels were measured in both groups at baseline. Adjusted odds ratios (OR) for the highest tertile of serum fructosamine compared to the lowest was 1.60 [95% confidence interval (CI), 0.95-2.73]. In premenopausal women, the OR was 1.58 (95% CI, 0.76-3.40) and in postmenopausal women, the OR was 1.60 (95% CI, 0.76-3.48). Serum fructosamine levels tended to be positively associated with breast cancer risk independent of menopausal status. (Cancer Epidemiol Biomarkers Prev 2005;14(1):271–4)

Introduction

There is increasing evidence that obesity and diabetes mellitus are associated with increased risk of breast carcinomas. Biological evidence provides support for a role of glucose and other factors related to glucose metabolism, such as insulin and C-peptide, in breast cancer development. It is known that glucose favors the selection of malignant cell clones and that the neoplastic cell extensively uses glucose for proliferation (1). Insulin has been shown to be a potent mitogenic agent (2). Insulin also induces a dose-dependent growth response in breast cancer cell lines and acts through the insulin receptor (3, 4). Furthermore, insulin may also play a role in tumor promotion by up-regulation of ovarian steroid secretion (5-7).

There is epidemiologic evidence supporting the relationship between abnormal glucose metabolism and breast cancer risk. There was an increase of breast cancer risk for women who had a diagnosis of diabetes mellitus at baseline in four prospective studies (8-11); however, a fifth did not corroborate the evidence. Furthermore, studies have shown a positive relationship between insulin and C-peptide levels and breast cancer incidence (12, 13). Additionally, variables related to insulin resistance, such as body mass index (BMI) and abdominal obesity have been related prospectively to breast cancer risk in postmenopausal women (14-16).

Given the evidence that supports a relationship between abnormal glucose metabolism and breast cancer risk, the measurement of markers of insulin resistance may help to identify women at high risk for breast cancer. Serum fructosamine, a product of protein glycation, may be suitable for the assessment of glucose metabolism in epidemiologic studies. The spontaneous, nonenzymatic condensation of glucose and proteins initially produces an unstable ketoamine, which is generally referred to as fructosamine due to its structural similarities to fructose (17). Glycated albumin usually accounts for 80% of the glycated serum proteins (18, 19). Serum fructosamine is more strongly correlated with habitual intake of sugar (r = 0.26, P = 0.05) than glycated hemoglobin (r = 0.001, P = 0.99). Thus, fructosamine can also be considered an index of the chronic exposure to sugar intake (20).

The purpose of our study was to investigate the association between fructosamine and breast cancer cases in a prospective study. The primary hypothesis of the present study was that prediagnostic serum fructosamine, as a marker of glucose metabolism, was associated with subsequent breast cancer.

Materials and Methods

Between June 1987 and June 1992, 10,786 healthy women, ages 35 to 69 years, residents of Varese province in northern Italy, participated in a prospective study of hormones, diet and breast cancer risk: the Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) study (20, 21). All members of the cohort were volunteers recruited from the general population. The total number of women recruited in the cohort represented ~7% of the general population of women in that age range in Varese province.

A major focus of the ORDET study was endogenous hormones and their relation to breast cancer risk. Therefore, several sources of hormone variability were controlled for by...
inclusion criteria and highly standardized conditions at blood drawing during the recruitment phase. Exclusion criteria included women with bilateral ovariectomy, those currently pregnant or breast-feeding, those on oral contraceptives, or hormone replacement therapy, or those affected by metabolic diseases and women with a previous history of cancer. At baseline, information on diet, reproductive history, family history of breast cancer, education, and occupational history were collected as well as anthropometric data.

After an average of 5.5 years of follow-up, the ORDET data were linked with the local Lombardy Cancer Registry (22, 23). These files were used to identify breast cancer cases. The regional municipal data of Varese residents was used to verify the vital status of the cohort members. Ten women were lost to follow-up, 37 women had been diagnosed with breast cancer before final enrollment in the cohort, and 4 were diagnosed with breast cancer in situ. Therefore, there were 10,735 women available for the study. Among these women, 89 died from causes other than breast cancer, and 144 were identified by the cancer registry as cases of invasive breast cancer (73 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 into the study.

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

Blood samples were collected after at least 12 hours of fasting between 7:30 and 9:00 a.m. from all participants at recruitment. 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 were done.

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. The laboratory personnel were blinded to case-control status. The control of analytic 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, IN). The intrabatch CV derived from the quality control serum included in the analytic runs was 2.5%. Fructosamine was determined using reagents, calibrators and controls from Sigma Diagnostics (St. Louis, MO) and application parameters for the Cobas Mira automated chemistry analyzer. The assay is a modification of the original method of Johnson and colleagues (24) where fructosamine (glycated serum protein) reduces nitroblue tetrazolium under alkaline conditions and forms a purple-colored formazan with an absorption maximum at 530 nm.

There were no stored serum specimens for 4 premenopausal breast cancer cases and 11 matched control subjects, and 7 postmenopausal cases and 18 controls. The final analysis included 69 premenopausal and 64 postmenopausal breast cancer cases and 265 premenopausal and 238 postmenopausal controls.

**Statistical Analysis.** Means and SD for serum glucose and fructosamine and for other risk factors were computed and compared for cases and control subjects with one-way ANOVA. In addition, we examined the difference between average fructosamine levels among smokers and nonsmokers. Pearson correlation coefficients were determined for the variables in the model and for serum glucose. The relationships between serum glucose and serum fructosamine were examined in premenopausal and postmenopausal controls using scatter plots. Serum fructosamine and BMI were collapsed into three and two categories, respectively, and these categories were used in the analyses. The cut-offs for serum fructosamine were based on the tertiles of the controls and the cut-off for BMI on the median of the controls to maintain the cut-off point used for BMI in the analysis of the original study. We estimated adjusted odds ratios and 95% confidence intervals (CI) for fructosamine using conditional logistic regression. We identified age, age at menarche, age at first birth, parity, BMI as potential covariates according to their potential biological relevance and logistic regression was used to control for these covariates. In the initial regression model, we examined all variables. We evaluated each covariate for confounding by removing each from the fully adjusted model. Age, age at menarche, age at first birth, parity, and BMI did not substantially modify the results. None of the potential covariates was a confounder of the association between breast cancer and fructosamine levels. Nevertheless, we included them in further analysis to provide fully adjusted estimates for comparison with those reported in the published literature, in particular, with the previous prospective cohort studies evaluating variables related to glucose metabolism (insulin and C-peptide) in relation to breast cancer risk. We also performed analyses after stratification by menopausal status.

**Results**

In Table 1, we report descriptive data on the study participants. Serum glucose levels were ~5% lower for premenopausal women than for postmenopausal women. Fasting glucose levels were significantly correlated with fructosamine levels among controls in the premenopausal women \((r = 0.129, P = 0.018)\) and postmenopausal women \((r = 0.3019, P < 0.0001)\). The correlation between fructosamine and glucose in postmenopausal women was stronger and this group of women on average had higher values for serum glucose as well. Figure 1 displays a scatter plot of glucose and fructosamine in controls.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of breast cancer cases and controls by menopausal status</th>
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<tbody>
<tr>
<td><strong>Premenopausal women</strong></td>
</tr>
<tr>
<td>Cases (n = 69), mean (± SD)</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>Age at menarche (y)</strong></td>
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<tr>
<td><strong>Age at first birth (y)</strong></td>
</tr>
<tr>
<td><strong>Number of children</strong></td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
</tr>
<tr>
<td><strong>Fructosamine (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>Smoking (yes/no)</strong></td>
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</tbody>
</table>

*One-way ANOVA \((P < 0.05)\): differences between cases and controls.
In Table 2, the descriptive data for the categories of fructosamine levels are shown. Cases were more likely to have elevated fructosamine levels than control subjects prior to diagnosis (43% of premenopausal cases and 41% of postmeno-
apausal cases were classified in the highest category).

Breast cancer risks in relation to categories of fructosamine are also shown in Table 2. Considering the group of women as a whole, women in the highest tertile of fructosamine had a nonsignificant 60% times higher risk of developing incident breast cancer (95% CI, 0.95-2.73). When we separated the sample in women who were in premenopausal and postmeno-
apausal status at recruitment, we obtained similar risk estimates: 1.58 (95% CI, 0.76-3.40) and 1.60 (CI, 0.76-3.48), respectively. All the CIs included unity and there was no evidence of a linear dose-effect relation (P for trend = 0.32). Adjustment for glucose did not alter the odds ratio estimates.

Discussion

We observed a 60% risk increase for breast cancer in women with high levels of fructosamine. Although the results failed to reach conventional levels of statistical significance, our results are important because this is the first study investigating serum fructosamine in relation to breast cancer risk. Our results confirm previous work in which we observed an association of fasting glucose and breast cancer risk in premenopausal women and in heavier postmenopausal women. Although in the latter group of women, the relation was not statistically significant (25). Thus, there is accumulating evidence suggesting a relationship between impaired glucose metabolism and the outcome of breast cancer. Fructosamine is a biomarker of habitual sugar intake, a risk factor for the development of hyperinsulinemic insulin resistance and type 2 diabetes (26). The availability of such a marker for prospective studies would enhance epidemiologic research in this area.

The spontaneous, nonenzymatic condensation of glucose and proteins initially produces an unstable ketoamine, which is referred to as fructosamine (16). Fructosamine, then, is the generic name for plasma protein ketoamines. Available literature indicates that short- to intermediate-term glycemic control is best reflected by glycated albumin (27, 28). For individuals with hemoglobin variants, assaying fructosamine may be a superior method compared with measuring glycated hemoglobin (29-31).

Limitations of this investigation warrant consideration. First, although the results from this study suggest that fructosamine may be predictive of breast cancer, the confidence limits included unity. Second, our results are based on direct determination of fructosamine and many factors may have affected the study results. We were not able to make albumin determinations due to limitations in serum availabil-

ity. Ohkawara et al. (32) measured fructosamine levels using extracted albumin and a fructosamine assay that depends on the potential of the glycated proteins to reduce nitroblue tetrazolium. In that study, the corrected albumin fructosamine values correlated more closely with fasting blood glucose levels (r = 0.735) than the serum fructosamine values corrected for albumin (r = 0.514; ref. 32). Although the correlation coefficient we observed in our study between serum glucose and fructosamine were significant, they were considerably lower compared with the study by Ohkawara and we did not account for albumin levels.

Another concern is the biological and technical variation of fructosamine (18). For instance, previous reports indicated that samples could be stored for several months at −20°C, but large changes have been observed in frozen samples (18). Prolonged storage at ultra-low temperatures (−196°C) prevents in vitro glycation of serum proteins (18). Ballard et al. (33) examined storage effects on human serum fructosamine concentration at 1 hour, 1 week, and at 6 months. These samples were stable over 6 months at −40°C and at −196°C (33). They concluded, however, that samples should not be kept longer than 1 week at +40°C, but could be kept for 6 months at −196°C (34). In our study, the samples were stored at −80°C until biochemical determination. However, case-control sets were matched on time since recruitment.

In addition to technical variation, other factors may contribute to variability in fructosamine levels. Some of these physiologic variables include prolonged bed rest, strenuous exercise, circadian variation, and diet (29). We did control for circadian differences as blood was collected at a specific time of day for all participants and all levels were fasting, but we were unable to control for the other factors. However, when we looked at serum fructosamine levels among all women, there was a significant difference between current smokers and nonsmokers, but the addition of smoking status, number of cigarettes smoked per day or peak number of cigarettes smoked did not change fructosamine risks when added to the regression model.

Premenopausal and postmenopausal women differed in fructosamine levels. This difference may be a reflection of differences in glucose levels we observed between these two groups of women. In addition, although levels were fasting, serum fructosamine levels will reflect intake from the past several weeks. As such, the level reflects usual dietary intake and not necessarily current intake. This could explain the differences in correlation between premenopausal and post-

menopausal groups. Perhaps, postmenopausal women have a less varied diet and less physical activity than premenopausal women and thus the fructosamine levels of postmenopausal women are more consistent with current glucose levels.

Table 2. Adjusted risks (odds ratios) and 95% CI by serum levels of fructosamine

<table>
<thead>
<tr>
<th>Fructosamine level</th>
<th>Cases/controls</th>
<th>Overall odds ratios* (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1.76*</td>
<td>26/129</td>
<td>1.00</td>
</tr>
<tr>
<td>1.76 to ≤1.88</td>
<td>50/201</td>
<td>1.19 (0.71-2.05)</td>
</tr>
<tr>
<td>&gt;1.88</td>
<td>55/174</td>
<td>1.60 (0.95-2.73)</td>
</tr>
</tbody>
</table>

*Adjusted for age, age at menarche, age at first birth, parity, and BMI.

†Reference category.
In conclusion, we found that serum fructosamine, as an indicator of glucose consumption, may be a predictor of breast cancer. The 60% increase in risk was similar for both premenopausal and postmenopausal women but the results failed to reach statistical significance. Nevertheless, further investigation of this biomarker of glucose metabolism is of interest as a potential additional mechanism explaining breast cancer etiology.

References
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