UNCLASSIFIED

AD NUMBER

ADB284009

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Jun 2002. Other requests shall be referred to US Army Medical Research and Materiel Comd., 504 Scott St., Fort Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, 27 Feb 2003

THIS PAGE IS UNCLASSIFIED

AD_____

Award Number: DAMD17-99-1-9327

TITLE: Insulin and Breast Cancer Risk

PRINCIPAL INVESTIGATOR: Paola C. Muti, M.D.
Teresa Quattrin
Jo L. Freudenheim

CONTRACTING ORGANIZATION: The Research Foundation of the State University of New York
Amherst, New York 14228

REPORT DATE: June 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Jun 02). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20021115 043

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-99-1-9327
Organization: The Research Foundation of the State University of New York

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Carole B Christian

10/15/02

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
June 2002

3. REPORT TYPE AND DATES COVERED
Final (1 Jun 99 - 31 May 02)

4. TITLE AND SUBTITLE
Insulin and Breast Cancer Risk

5. FUNDING NUMBERS
DAMD17-99-1-9327

6. AUTHOR(S)
Paola C. Muti, M.D.
Teresa Quattrin
Jo L. Freudenheim

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Research Foundation of the State University of New York
Amherst, New York 14228

E-Mail: muti@acsu.buffalo.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Jun 02). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The present study analyzed the hypothesis that serum glucose, insulin levels, and insulin-like growth factor (IGF)-I pattern are associated with breast cancer using a nested case-control study. Between 1987 and 1992, 10,786 women aged 35-69 were recruited in a prospective study in Italy. At recruitment, blood samples were collected after 12 hours fast between 7:30 and 9:00 AM from all 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, BMI, 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], p for trend 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), p for trend 0.14, while the adjusted RR of the highest quartile of IGF-I was 3.1 (95% CI 1.1 – 8.6), p for trend 0.01. Increased levels of IGFBP-3 were related to breast cancer risk: the adjusted RR for the highest quartile was 2.1 (95% CI 0.95 – 4.75), p for trend 0.02. In postmenopausal women, none of the variables was associated with breast cancer risk.

14. SUBJECT TERMS
breast cancer, glucose, insulin, IGF-I, premenopausal women, serum glucose, relative risk (RR)

15. NUMBER OF PAGES
62

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover.....

SF 298.....2

Introduction.....4

Body.....5

Key Research Accomplishments.....14

Reportable Outcomes.....15

Conclusions.....15

References.....18

Appendices.....32

INTRODUCTION

There is biological 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. 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 utilize 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 upregulation of ovarian steroid secretion (6,7). Intraportal insulin levels influence IGF-1 bioavailability (8). IGF-I is a small peptide (about 7,500 DA) with significant structural homology with proinsulin and insulin (9), and is highly regulated by growth hormone (GH) (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 measured 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-1 pattern were associated with breast cancer.

BODY

Between June 1987 and June 1992, 10,786 healthy women, aged 35 to 69 years, residents of Varese province, Northern Italy, participated in a prospective study of hormones, diet, and breast cancer risk: the H**OR**mones and **D**iet in the **ETi**ology of breast cancer prospective cohort study (ORDET) (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 approximately 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. 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, four 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, four 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, seven 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 hours fasting between 7:30 and 9:00 AM from all 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 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 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, Texas), with an intrabatch coefficient of variation of 5.2%. IGF-I, free IGF-I and IGFBPs were determined by double-antibody, immunoradiometric assay (IRMA) (Diagnostic Systems

Laboratories, Inc., Webster, Texas). 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 one-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 one-year apart in the same month, on the same day of the month, and at the same hour and minute of the day between 7:30 and 9:00 AM 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 standard deviations 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. Due to skewed distributions, serum glucose, insulin, IGFBP-1 and IGFBP-2 and free IGF-I were log transformed for all analyses. Quartiles of exposure for the relevant variables were calculated based on the distribution of the controls. We estimated relative risks (RR) (26) and 95% confidence intervals (CI) using conditional logistic regression. For adjusted analyses, we controlled for age, body mass index (BMI), waist-to-hip ratio and social and economic status (SES), 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 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 intraclass correlation coefficient (ICC) (27).

Results

The ICCs and the lower limit of the 95% confidence interval, reported in parenthesis, 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 not systematic differences in reliability across all 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 notably 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).

In Table 1, we report descriptive data on the study participants. Serum glucose and insulin levels were approximately 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 positively correlated 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 ($r=0.37$, $p<.0.001$) 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, 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 the confidence intervals included unity and there was no evidence of a linear dose-effect relation (p for trend = 0.32). Breast cancer risk increased 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 and after age of 48 years, 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 characterized by very large confidence intervals (Tables 4 and 5).

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 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 (three breast cancer and nine control subjects) showed serum fasting glucose levels at baseline that were higher than 126 mg/dL, the threshold value for the definition of clinical diabetes (32). We repeated all analyses with the exclusion of these 33 subjects and the controls matched to the seven 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 previously observed 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 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 relative risks 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, 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 6). 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).

During the conduct of the study, we did not observe specific problems in accomplishing any of the task.

KEY ACCOMPLISHMENTS

1) The most interesting findings of the study is the association of fasting glucose, an indicator of insulin resistance and impaired glucose metabolism in population based studies, with breast cancer risk in premenopausal women.

2) We also confirm previous epidemiological observations that elevated circulating IGF-I precedes breast cancer occurrence in premenopausal women (Hankinson S et al, 1998; Toniolo P et al., 2000).

3) We also observe a positive association of IGFBP-3 and breast cancer.

4) In postmenopausal women, glucose, and IGF-I pattern are not associated with breast cancer. However, we observe a tendency toward a potential association of these variables only in the the sub-group of postmenopausal women at high BMI.

This is the first time that fasting glucose has been identified as strong predictor of breast cancer. The potential implication of this outcome, if confirmed by future studies, will be of huge relevance for breast cancer research, prevention and therapy.

REPORTABLE OUTCOMES:

The present outcomes have been presented at the following **meetings**:

1. **Muti P**, Quattrin T, Misciagna G, Krog V, Micheli A, Browne R, Berrino F. Fasting *serum glucose and insulin and breast cancer risk in premenopausal and postmenopausal women: the ORDET study.* American Association Cancer Research, New Orleans, LA, USA, March 2001

2. Micheli A, **Muti P,** Krogh V, Mugno E, Sieri S, Pala V, Meneghini E, Cifala' A, Berrino F. *ORDET: fattori di rischio in donne in postmenopausal in uno studio prospettico.* XXVI Reunion Du Groupe Pour L'epidemiologie Et L'enregistrement Du Cancer Dans Les Pays De Langue Latine, Neuchâtel (Switzerland), May 24-25, 2001

1) **Muti P.** "*Endogenous Hormones and Breast Cancer Risk*" NCI – Estrogen and Cancer Group- Washington, USA, July 2002

2) **Muti P,** "*Endogenous sex steroids and glucose metabolism and breast cancer risk*" Department of Gynecology-Obstetrics, University at Buffalo, NY, USA, 2002

3) **Muti P,** "*Glucose and Breast Cancer*" Lecture for "Advances in Human Nutrition", Nutrition Division, Dept. of Physical Therapy, Exercise and Nutrition Sciences, University of Buffalo, November, 2001

4) **Muti P,** "*Metabolic factors in breast cancer etiology*" Roswell Park Cancer Institute, Buffalo, NY, June, 2001

The attached **paper** has been send for publication to Cancer Epidemiology, Biomarkers and Prevention:
Muti P, Quattrin T, Grant B, Krogh V, Micheli A, Schünemann HJ, Ram M, Freudenheim J, Sieri S, Trevisan M, Berrino F Fasting Glucose is a Risk Factor For Breast Cancer: A Prospective Study.

CONCLUSION

The most interesting findings of the present study is the association of fasting glucose with breast cancer risk in premenopausal women. We previously found in this population that abdominal adiposity was related to breast cancer risk only in premenopausal women (16). In our study, the association of serum fasting glucose appeared to be independent

of the levels of insulin and IGF-I since 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 counterregulatory 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 further 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).

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-1 and breast cancer only in premenopausal women (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 (52, 53). Thus, free IGF-I may exert a permissive

effect towards 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, 54). One of the proposed functions of IGFBPs is to increase the half-life of IGFs in circulation (52, 53). Thus, IGFBP-3 may enhance the action of IGF-I by protecting it from degradation (55). 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 (56). 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 (57). There is evidence that estrogens increase the levels of cellular IGF-I and that IGF-I upregulates responses to estrogen at the receptor level (58). In our study, most of the factors related to glucose metabolism and IGF-I pattern were associated with an increased risk of breast cancer only 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 (59) and by the increased availability of estrogens due to the aromatization of androgens in adipose tissue.

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 mainly restricted to premenopausal women. Further 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.

REFERENCES

1) Warburg O. On the origin of cancer cells. Science 1956; 123:309-14.

2) Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Bioch Science 1999; 24: 68-72.

3) Milazzo G, Giorgino F, Damante F. et al. Insulin receptor expression and function in human breast cancer cell lines. Cancer Res. 1992; 52:3924-30.

4) Cullen KJ, Yee D, Sly WS, Perdue J, Hampton B, Lippman ME, Rosen N. Insulin like growth factor receptor expression and function in human breast cancer. Cancer Res 1990; 50:48-53.

5) Osborne CK, Clemmons DR, Arteaga CI. Regulation of breast cancer growth by insulin-like growth factors. J Steroid Biochem Mol Biol 1990; 37:805-9.

6) Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC The insulin-related ovarian regulatory system in health and disease. Endocrine Reviews 1999; 20:535-582.

7) Nestler JE, Strauss JE. Insulin as an effector of human ovarian and adrenal steroid metabolism. Endocrinol Metab Clin North Am. 1991; 20:807-23

8) Lee PD, Giudice LC, Conover CA, Powell DR Insulin growth factor binding protein-1: recent finding and new directions. Proc Soc Rxp Biol Med 1997; 216:319-357

9) Lewitt MS. Role of the insulin-like growth factors in the endocrine control of glucose homeostasis. Diabetes Res Clin Pract 1994; 23:3-15.

10) Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 1995; 16:3-34.

11) Clemmons DR, Underwood LE. Nutritional regulation of IGF-I and IGF binding proteins. Ann Rev Nutr 1991; 11:393-412.

12) Weiderpass E, Gridley G, Persson I, Nyren O, Ekbon A, Adami HO. Risk of endometrial and breast cancer in patient with diabetes mellitus. Int J Cancer Inst 1997; 71: 360-3.

13) Goodman MT, Cologne JB, Moriwaki H, Vaeth M, Mabuchi K. Risk factors for primary breast cancer in Japan: 8-year follow-up of atomic bomb survivors. Prev Med 1997; 26:144-53.

14) Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998; 351:1393-6.

15) Toniolo P. Bruning PF. Akhmedkhanov A. Bonfrer JM. Koenig KL. Lukanova A. Shore RE. Zeleniuch-Jacquotte A. Serum insulin-like growth factor-I and breast cancer. International Journal of Cancer. 2000; 88:828-32.

16) Muti P, Stanulla M, Micheli A, et al. Markers of Insulin resistance and sex steroid activity in relation to breast cancer: a prospective analysis of abdominal adiposity, sebum production and hirsutism.. Cancer, Causes and Control 2000; 11:721-30.

17) Sonnenschein E, Toniolo P, Terry MB, Bruning PF, Kato I, Koenig KL, Shore RE. Body fat distribution and obesity in pre- and postmenopausal breast cancer. International Journal of Epidemiology. 1999; 28(6):1026-31.

18) Huang Z, Willett WC., Colditz GA, Hunter DJ, Manson JE, Rosner B, Speizer FE, Hankinson SE. Waist circumference, waist:hip ratio, and risk of breast cancer in the Nurses' Health Study. American Journal of Epidemiology. 1999; 150(12):1316-24.

19) Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, Hennekens CH. Rosner B. Speizer FE. Willett WC. Dual effects of weight and weight gain on breast cancer risk. JAMA. 1997; 278(17):1407-11.

20) Berrino F, Pisani P, Muti P, Crosignani P, Panico S, Pierotti M, Secreto G, Totis A, Fissi R, Mazzoleni Prospective study on hormones and diet in the etiology of breast cancer In: Diet, hormones and cancer: methodological issues for prospective studies, E. Riboli and R. Saracci eds., IARC Technical Report No. 4, 1988.

21) Berrino F, Muti P, Micheli A, Bolelli GF, Krogh V, Sciajno R, Pisani P, Panico S, Secreto G.Serum sex steroids levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996; 88:291-6.

22) XIII Censimento Generale della Popolazione. Dati sulle caratteristiche strutturali della popolazione e delle abitazioni – ISTAT, Roma 1993

23) Cancer Incidence in Five Continents Volume IV Waterhouse J, Muir C, Shanmugaratnam K (eds) IARC Scientific Publications, 1982.

24) Cancer Incidence in Five Continents Volume VII Parlin DM, Whelan J, Ferlay L, Raymond L, Young J (eds) IARC Scientific Publications, 1997.

25) Muti P, Trevisan M, Micheli A, Krogh V, Bolelli G.F, Sciajno R, and Berrino F. Reliability of serum hormones in premenopausal and in postmenopausal women over a one year period. Cancer Epidemiology, Biomarkers & Prevention 1996; 5:917-922.

26) Kleinbaum D.G, Kupper L.L, Morgenstern H. (1986) Measures of association. In: Kleinbaum D.G., Kupper L.L., Morgenstern H., eds, Epidemiologic Research New York: Van Nostrand Reinhold Company, pp.146-147.

27) Fleiss JL. Reliability of measurement (1986) In: Fleiss JL, ed. The Design and Analysis of Clinical Experiments. New York: NY John Wiley & Sons, pp. 1-14 .

28) Clavel-Chapelon F. E3N-EPIC Group. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. British Journal of Cancer. 86(5):723-7, 2002.

29) De Waard F. Breast cancer incidence and nutritional status with particular reference to body weight and height. Cancer Res 1975; 35:3351-6.

30) Swanson CA, Brinton LA, Taylor PR, Licitra LM, Ziegler RG, Schairer C. Body size and breast cancer risk assessed in women participating in the Breast Cancer Detection Demonstration Project. Am J Epidemiol 1989; 130:1133-41.

31) 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. American Journal of Epidemiology. 2000; 152(6):514-27.

32) Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000; 23 (suppl):s4-14.

33) Schoen RE, Tangen CM, Kuller LH, Burke GL. Cushman M. Tracy RP. Dobs A. Savage PJ. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999; 91:1147-54.

34) Manjer J. Kaaks R. Riboli E. Berglund G. Risk of breast cancer in relation to anthropometry, blood pressure, blood lipids and glucose metabolism: a prospective study within the Malmo Preventive Project. European Journal of Cancer Prevention. 2001; 10(1):33-42.

35) Rothman DL, Magnusson I, Katz LD. Quantification of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR Science 1991, 254:573-576.

36) Rizza RA, Mandarino L, Gerich JE Dose-response characteristics for the effects of insulin on production and utilization of glucose in man Am J Physiology 1981; 240:630-639.

37) Deibert DC, De Fronzo RA Epinephrine induced insulin resistance in man, J Clin Invest 1980, 65:717-721.

38) MacGorman LR, Rizza RA, Gerich JE Physiological concentrations of growth hormone exert insulin and anti-insulin antagonist effects on both hepatic and extrahepatic tissues in man J Clin Endocrinol Metab 1981; 53:556-559

39) Sipe HJ, Jordan SJ, Hanna PM, Mason RP. The metabolism of 17β-estradiol by lactoperoxidase: a possible source of oxidative stress in breast cancer. Carcinogenesis 1994; 15: 2637-43.

40) Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radicals and Biology and Medicine 1990, 8:583-99.

41) Dandona P, Thusu K, Snyder B, Makowski J, Armstrong D, Nicotera T. Oxidative damage o DNA in diabetes mellitus. Lancet 1996; 347: 444-5.

42) Kitara M, Eyre HJ, Lynch RE, Rallison ML, Hill HR. Metabolic activity of diabetics' monocytes. Diabetes 1980; 29:251-6 .

43) Armstrong D, Al-Awadi F. Lipid peroxidation and retinopathy in steptozotocin induced diabetes. Free Radic Biol Med 1991; 11:433-6.

44) Kazer RR. Insulin resistance, insulin-like growth factor-I and breast cancer: a hypothesis. Int J Cancer 1995; 62:403-6.

45) Kim YI. Diet, lifestyle and colonrectal cancer: Is hyperinsulinemia the missing link? Nutrition Reviews 1999; 56:275-9.

46) Kaaks R. Nutrition, hormones, and breast cancer: Is insulin the missing link? Cancer Causes and Control 1996; 7:605-25.

47) Shafie SM, Gibson SL, Hilf R. Effect of insulin and estrogen on hormone binding in R3230AC mammary adenocarcinoma. Cancer Res 1977; 37:4641-9.

48) Pekonen F, Partanen S, Makinen T, Rutanen EM. Receptors for epidermal growth factor and insulin-like growth factor-I and their relation to steroid receptors in human breast cancer. Cancer Res 1988; 48:1343-7.

49) Papa V. Milazzo G. Goldfine ID. Waldman FM. Vigneri R. Sporadic amplification of the insulin receptor gene in human breast cancer. J Endocrinol Invest 1997; 20:531-6.

50) Webster NJG, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL. Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression. Cancer Res 1996; 56:2781-8

51) Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 2000; 92:1472-89.

52) Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16:3-34

53) Rajah R, Katz L, Nunn S, Solberg P, Beers T, Cohen P. Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth. Progr Growth Factor Res 1995; 6:273-84.

54) Conover CA. Regulation and physiological role of insulin-like growth factor binding proteins. Endocr J 1996; 43 (suppl):S 43-8.

55) Rocha RL, Hilsenbeck SG, Jackson JG, Van Den Berg CL, Weng C, Lee AV. Yee D. Insulin-like growth factor binding protein-3 and insulin receptor substrate-1 in breast cancer: correlation with clinical parameters and disease-free survival. Clin Cancer Res 1997; 3:103-9.

56) Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, Ng L, Simpson DM. Rosenfeld RG. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int J Biochem Cell Biol 1996; 28:619-37.

57) Yen SCC. The biology of the menopause. J Reprod Med 1977; 18:287-96.

58) Chaudhuri PK. Chaudhuri B. Patel N. Modulation of estrogen receptor by insulin and its biologic significance. Arch Surg 1986; 121:1322-5.

59) De Perola G, Cospite MR, Giaculli VA, Giorgino F, Garruti G, Cignarelli M, Giorgino R. Insulin-like growth factor I and Dehydroepiandrosterone sulphate in obese women. Int J Obesity 1993; 17: 481-3

60) Giampietro O, Navalesi R, Buzzigoli G, Boni C, Benzi L Decrease in plasma glucose concentration during storage at –20 C, 1980, 25:1710-1712.

61) Chan AYW, Swaminathan T, Cockram CS Effectiveness of sodium fluoride as a preservative of glucose in blood Clin Chem 1989; 35:315-317

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)*
Glucose (mg/dL)	81.9 (18.9)	78.6 (10.8)*	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)*	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

* One way ANOVA ($P < .05$): differences between cases and controls.

Table 2 Estimated Risks (RR) of Premenopausal Breast Cancer and 95% confidence limits by serum levels of fasting glucose, insulin, insulin-like-growth factor (IGF)-I, and IGF Binding Proteins (IGFBPs).

	I	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)	**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)	**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)	**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)	**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)	**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)	**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)	**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	

I= Reference category

• Adjusted for age, BMI, age at menarche, age at first birth, parity.

Table 3 Estimated Risks (RR) of Postmenopausal Breast Cancer and 95% confidence limits by serum levels of fasting glucose, insulin, insulin-like-growth factor (IGF)-I, and IGF Binding Proteins (IGFBPs).

	I	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)	**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 (mg/dl)	≤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)	**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)	**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)	**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)	**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)	**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)	**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	

I= Reference category

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

Table 4. PREMENOPAUSAL WOMEN CHARACTERIZED BY AN AGE AT DIAGNOSIS LESS THAN 48 YEARS (36 cases and 138 control subjects).
Estimated risks (RR) of breast cancer risk and 95% confidence limits (95%CI) by serum levels of fasting glucose, insulin, insulin growth factor (IGF)-1 and IGF binding proteins (IGFBPs).

Variables	1 Tertile RR (95% CI)	2 Tertile RR (95% CI)	3 Tertile RR (95% CI)
Glucose	1.00	1.43 (0.55-3.73)	2.30 (0.74-7.09)
Insulin	1.00	1.26 (0.49-3.23)	0.87 (0.27-2.81)
IGF-1	1.00	1.51 (0.53-4.29)	1.52 (0.50-4.60)
Free IGF-1	1:00	0.55 (0.17-1.71)	0.74 (0.23-2.34)
IGFBP-1	1:00	0.52 (0.17-1.58)	1.23 (0.38-3.95)
IGFBP-2	1:00	1.04 (0.38-2.78)	1.04 (0.33-3.26)
IGFBP-3	1:00	1.04 (0.39-2.74)	1.12 (0.41-3.06)

Table 5. PREMENOPAUSAL WOMEN CHARACTERIZED BY AN AGE AT DIAGNOSIS EQUAL-MORE THAN 48 YEARS (33 cases and 127 contro subjects). Estimated risks (RR) of breast cancer risk and 95% confidence limits by serum levels of fasting glucose, insulin, insulin growth factor (IGF)-1 and IGF binding proteins (IGFBPs).

Variables	1 Tertile RR (95% C I)	2 Tertile RR (95% CI)	3 Tertile RR (95% CI)
Glucose	1.00	0.78 (0.21-2.85)	4.20 (1.12-15.72)
Insulin	1.00	2.00 (0.50-7.97)	4.34 (0.97-19.35)
IGF-1	1.00	2.46 (0.65-9.29)	15.43 (3.25-73.19)
Free IGF-1	1:00	2.24 (0.63-7.92)	4.97 (1.33-18.54)
IGFBP-1	1:00	0.82 (0.25-2.67)	0.27 (0.06-1.18)
IGFBP-2	1:00	0.38 (0.11-1.31)	0.61 (0.16-2.25)
IGFBP-3	1:00	1.85 (0.56-6.02)	2.70 (0.68-10.70)

Table 6 Estimated risk of Postmenopausal Breast Cancer associated with glucose metabolism and IGF-I factors by strata of BMI

	LOW BMI ≤26			*HIGH BMI* >26			
Tertiles of Variables	I	II	III	I	II	III	P•
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	

• P values for interactive terms of each variable with BMI

Point estimates were adjusted for age and reproductive variables

APPENDICES

Fasting Glucose is a Risk Factor For Breast Cancer: A Prospective Study

Paola Muti, M.D.,[1,2], Teresa Quattrin, M.D.[3], Brydon Grant, M.D.[4,5], Vittorio Krogh, M.D.[2], Andrea Micheli, Ph.D[2], Holger J. Schünemann, M.D.[1,4], Malathi Ram, Ph.D[1], Jo L. Freudenheim, Ph.D[1], Sabina Sieri, Ph.D[2], Maurizio Trevisan, M.D.[1], Franco Berrino, M.D.[2]

1-Department of Social and Preventive Medicine, University at Buffalo, State University of New York

2-Epidemiology Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy

3-Department of Pediatrics, Center for Diabetes, University at Buffalo, State University of New York

4- Department of Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, University at Buffalo

5- Section of Pulmonary, Critical Care and Sleep Medicine, Veterans Administration Medical Center, Buffalo, NY 14215

Running title: Serum glucose and subsequent breast cancer risk

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 insulin-like growth factor (IGF)-I pattern are associated with breast cancer using a nested case-control study. Between 1987 and 1992, 10,786 women aged 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 12 hours fast between 7:30 and 9:00 AM from all 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, BMI, 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], p for trend 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), p for trend 0.14, while the adjusted RR of the highest quartile of IGF-I was 3.1 (95% CI 1.1 – 8.6), p for trend 0.01. Increased levels of IGFBP-3 were related to breast cancer risk: the adjusted RR for the highest quartile was 2.1 (95% CI 0.95 – 4.75), p for trend 0.02. In postmenopausal women, none of the variables was associated with breast cancer risk.

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

Introduction

There is biological 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. 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 utilize 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 upregulation of ovarian steroid secretion (6,7). Intraportal insulin levels influence IGF-1 bioavailability (8). IGF-I is a small peptide (about 7,500 DA) with significant structural homology with proinsulin and insulin (9), and is highly regulated by growth hormone (GH) (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 measured 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-1 pattern were associated with breast cancer.

Material and Methods

Between June 1987 and June 1992, 10,786 healthy women, aged 35 to 69 years, residents of Varese province, Northern Italy, participated in a prospective study of hormones, diet, and breast cancer risk: the HORmones and Diet in the ETiology of breast cancer prospective cohort study (ORDET) (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 approximately 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. 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, four 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, four 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, seven 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 hours fasting between 7:30 and 9:00 AM from all 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 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 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, Texas), with an intrabatch coefficient of variation of 5.2%. IGF-I, free IGF-I and IGFBPs were determined by double-antibody, immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Inc., Webster, Texas). 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 one-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 one-year apart in the same month, on the same day of the month, and at the same hour and minute of the day between 7:30 and 9:00 AM 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 standard deviations 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. Due to skewed distributions, serum glucose, insulin, IGFBP-1 and IGFBP-2 and free IGF-I were log transformed for all analyses. Quartiles of exposure for the relevant variables were calculated based on the distribution of the controls. We estimated relative risks (RR) (26) and 95% confidence intervals (CI) using conditional logistic regression. For adjusted analyses, we controlled for age, body mass index (BMI), waist-to-hip ratio and social and economic status (SES), 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 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 intraclass correlation coefficient (ICC) (27).

Results

The ICCs and the lower limit of the 95% confidence interval, reported in parenthesis, 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 not systematic differences in reliability across all 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 notably 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).

In Table 1, we report descriptive data on the study participants. Serum glucose and insulin levels were approximately 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 positively correlated 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 ($r=0.37$, $p<.0.001$) 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, 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 the confidence intervals included unity and there was no evidence of a linear dose-effect relation (p for trend = 0.32). Breast cancer risk increased 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 and after age of 48 years, 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 characterized by very large confidence intervals (data not shown).

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 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 (three breast cancer and nine control subjects) showed serum fasting glucose levels at baseline that were higher than 126 mg/dL, the threshold value for the definition of clinical diabetes (32). We repeated all analyses with the exclusion of these 33 subjects and the controls matched to the seven 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 previously observed 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 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 relative risks 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, 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. We previously found in this population that abdominal adiposity was related to breast cancer risk only in premenopausal women (16). Schoen and co-authors reported that fasting glucose was associated with colonrectal cancer,

another type of cancer whose etiology has been related to impaired fasting glucose and hyperinsulinemic insulin resistance (33). Another prospective 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 since 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 counterregulatory 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 further 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 over-expressed 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-1 and breast cancer only in premenopausal women (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 (52, 53). Thus, free IGF-I may exert a permissive effect towards 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, 54). One of the proposed functions of IGFBPs is to increase the half-life of IGFs in circulation (52, 53). Thus, IGFBP-3 may enhance the action of IGF-I by protecting it from degradation (55). 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 (56). 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 (57). There is evidence that estrogens increase the levels of cellular IGF-I and that IGF-I upregulates responses to estrogen at the receptor level (58). In our study, most of the factors related to glucose metabolism and IGF-I pattern were associated with an increased risk of breast cancer only 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 (59) and by the increased availability of estrogens due to the aromatization of androgens in adipose tissue.

Limitations of this investigation warrant consideration. The results of this study are based on a relatively small number of 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 (60, 61). 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 mainly restricted to premenopausal women. Further 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.

The ORDET cohort study is being financed by the Italian League against Cancer (Milan Section) and the Italian Ministry of Health with the contribution of the Italian National Research Council (P.F. A.C.R.O. number 93.02272, PF 39). The present phase of case-control studies has been supported by the Italian Association for Cancer Research (AIRC). The present case-control analyses has been supported by the USA Department of Defense, Medical Research and Material Command Breast Cancer Research Program, Grant Number DAMD 179919327.

References

1) Warburg O. On the origin of cancer cells. Science 1956; 123:309-14.

2) Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Bioch Science 1999; 24: 68-72.

3) Milazzo G, Giorgino F, Damante F. et al. Insulin receptor expression and function in human breast cancer cell lines. Cancer Res. 1992; 52:3924-30.

4) Cullen KJ, Yee D, Sly WS, Perdue J, Hampton B, Lippman ME, Rosen N. Insulin like growth factor receptor expression and function in human breast cancer. Cancer Res 1990; 50:48-53.

5) Osborne CK, Clemmons DR, Arteaga CI. Regulation of breast cancer growth by insulin-like growth factors. J Steroid Biochem Mol Biol 1990; 37:805-9.

6) Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC The insulin-related ovarian regulatory system in health and disease. Endocrine Reviews 1999; 20:535-582.

7) Nestler JE, Strauss JE. Insulin as an effector of human ovarian and adrenal steroid metabolism. Endocrinol Metab Clin North Am. 1991; 20:807-23

8) Lee PD, Giudice LC, Conover CA, Powell DR Insulin growth factor binding protein-1: recent finding and new directions. Proc Soc Rxp Biol Med 1997; 216:319-357

9) Lewitt MS. Role of the insulin-like growth factors in the endocrine control of glucose homeostasis. Diabetes Res Clin Pract 1994; 23:3-15.

10) Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 1995; 16:3-34.

11) Clemmons DR, Underwood LE. Nutritional regulation of IGF-I and IGF binding proteins. Ann Rev Nutr 1991; 11:393-412.

12) Weiderpass E, Gridley G, Persson I, Nyren O, Ekbon A, Adami HO. Risk of endometrial and breast cancer in patient with diabetes mellitus. Int J Cancer Inst 1997; 71: 360-3.

13) Goodman MT, Cologne JB, Moriwaki H, Vaeth M, Mabuchi K. Risk factors for primary breast cancer in Japan: 8-year follow-up of atomic bomb survivors. Prev Med 1997; 26:144-53.

14) Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998; 351:1393-6.

15) Toniolo P. Bruning PF. Akhmedkhanov A. Bonfrer JM. Koenig KL. Lukanova A. Shore RE. Zeleniuch-Jacquotte A. Serum insulin-like growth factor-I and breast cancer. International Journal of Cancer. 2000; 88:828-32.

16) Muti P, Stanulla M, Micheli A, et al. Markers of Insulin resistance and sex steroid activity in relation to breast cancer: a prospective analysis of abdominal adiposity, sebum production and hirsutism.. Cancer, Causes and Control 2000; 11:721-30.

17) Sonnenschein E, Toniolo P, Terry MB, Bruning PF, Kato I, Koenig KL, Shore RE. Body fat distribution and obesity in pre- and postmenopausal breast cancer. International Journal of Epidemiology. 1999; 28(6):1026-31.

18) Huang Z, Willett WC., Colditz GA, Hunter DJ, Manson JE, Rosner B, Speizer FE, Hankinson SE. Waist circumference, waist:hip ratio, and risk of breast cancer in the Nurses' Health Study. American Journal of Epidemiology. 1999; 150(12):1316-24.

19) Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, Hennekens CH. Rosner B. Speizer FE. Willett WC. Dual effects of weight and weight gain on breast cancer risk. JAMA. 1997; 278(17):1407-11.

20) Berrino F, Pisani P, Muti P, Crosignani P, Panico S, Pierotti M, Secreto G, Totis A, Fissi R, Mazzoleni Prospective study on hormones and diet in the etiology of breast cancer In: Diet, hormones and cancer: methodological issues for prospective studies, E. Riboli and

R. Saracci eds., IARC Technical Report No. 4, 1988.

21) Berrino F, Muti P, Micheli A, Bolelli GF, Krogh V, Sciajno R, Pisani P, Panico S, Secreto G.Serum sex steroids levels after menopause and subsequent breast cancer. J Natl Cancer Inst 1996; 88:291-6.

22) XIII Censimento Generale della Popolazione. Dati sulle caratteristiche strutturali della popolazione e delle abitazioni – ISTAT, Roma 1993

23) Cancer Incidence in Five Continents Volume IV Waterhouse J, Muir C, Shanmugaratnam K (eds) IARC Scientific Publications, 1982.

24) Cancer Incidence in Five Continents Volume VII Parlin DM, Whelan J, Ferlay L, Raymond L, Young J (eds) IARC Scientific Publications, 1997.

25) Muti P, Trevisan M, Micheli A, Krogh V, Bolelli G.F, Sciajno R, and Berrino F. Reliability of serum hormones in premenopausal and in postmenopausal women over a one year period. Cancer Epidemiology, Biomarkers & Prevention 1996; 5:917-922.

26) Kleinbaum D.G, Kupper L.L, Morgenstern H. (1986) Measures of association. In: Kleinbaum D.G., Kupper L.L., Morgenstern H., eds, Epidemiologic Research New York: Van Nostrand Reinhold Company, pp.146-147.

27) Fleiss JL. Reliability of measurement (1986) In: Fleiss JL, ed. The Design and Analysis

of Clinical Experiments. New York: NY John Wiley & Sons, pp. 1-14 .

28) Clavel-Chapelon F. E3N-EPIC Group. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. British Journal of Cancer. 86(5):723-7, 2002.

29) De Waard F. Breast cancer incidence and nutritional status with particular reference to body weight and height. Cancer Res 1975; 35:3351-6.

30) Swanson CA, Brinton LA, Taylor PR, Licitra LM, Ziegler RG, Schairer C. Body size and breast cancer risk assessed in women participating in the Breast Cancer Detection Demonstration Project. Am J Epidemiol 1989; 130:1133-41.

31) 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. American Journal of Epidemiology. 2000; 152(6):514-27.

32) Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000; 23 (suppl):s4-14.

33) Schoen RE, Tangen CM, Kuller LH, Burke GL. Cushman M. Tracy RP. Dobs A. Savage

PJ. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999; 91:1147-54.

34) Manjer J. Kaaks R. Riboli E. Berglund G. Risk of breast cancer in relation to anthropometry, blood pressure, blood lipids and glucose metabolism: a prospective study within the Malmo Preventive Project. European Journal of Cancer Prevention. 2001; 10(1):33-42.

35) Rothman DL, Magnusson I, Katz LD. Quantification of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR Science 1991, 254:573-576.

36) Rizza RA, Mandarino L, Gerich JE Dose-response characteristics for the effects of insulin on production and utilization of glucose in man Am J Physiology 1981; 240:630-639.

37) Deibert DC, De Fronzo RA Epinephrine induced insulin resistance in man, J Clin Invest 1980, 65:717-721.

38) MacGorman LR, Rizza RA, Gerich JE Physiological concentrations of growth hormone exert insulin and anti-insulin antagonist effects on both hepatic and extrahepatic tissues in man J Clin Endocrinol Metab 1981; 53:556-559

39) Sipe HJ, Jordan SJ, Hanna PM, Mason RP. The metabolism of 17β-estradiol by lactoperoxidase: a possible source of oxidative stress in breast cancer. Carcinogenesis 1994; 15: 2637-43.

40) Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radicals and Biology and Medicine 1990, 8:583-99.

41) Dandona P, Thusu K, Snyder B, Makowski J, Armstrong D, Nicotera T. Oxidative damage o DNA in diabetes mellitus. Lancet 1996; 347: 444-5.

42) Kitara M, Eyre HJ, Lynch RE, Rallison ML, Hill HR. Metabolic activity of diabetics' monocytes. Diabetes 1980; 29:251-6 .

43) Armstrong D, Al-Awadi F. Lipid peroxidation and retinopathy in steptozotocin induced diabetes. Free Radic Biol Med 1991; 11:433-6.

44) Kazer RR. Insulin resistance, insulin-like growth factor-I and breast cancer: a hypothesis. Int J Cancer 1995; 62:403-6.

45) Kim YI. Diet, lifestyle and colonrectal cancer: Is hyperinsulinemia the missing link? Nutrition Reviews 1999; 56:275-9.

46) Kaaks R. Nutrition, hormones, and breast cancer: Is insulin the missing link? Cancer Causes and Control 1996; 7:605-25.

47) Shafie SM, Gibson SL, Hilf R. Effect of insulin and estrogen on hormone binding in R3230AC mammary adenocarcinoma. Cancer Res 1977; 37:4641-9.

48) Pekonen F, Partanen S, Makinen T, Rutanen EM. Receptors for epidermal growth factor and insulin-like growth factor-I and their relation to steroid receptors in human breast cancer. Cancer Res 1988; 48:1343-7.

49) Papa V. Milazzo G. Goldfine ID. Waldman FM. Vigneri R. Sporadic amplification of the insulin receptor gene in human breast cancer. J Endocrinol Invest 1997; 20:531-6.

50) Webster NJG, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL. Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression. Cancer Res 1996; 56:2781-8

51) Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 2000; 92:1472-89.

52) Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16:3-34

53) Rajah R, Katz L, Nunn S, Solberg P, Beers T, Cohen P. Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth. Progr Growth Factor Res 1995; 6:273-84.

54) Conover CA. Regulation and physiological role of insulin-like growth factor binding proteins. Endocr J 1996; 43 (suppl):S 43-8.

55) Rocha RL, Hilsenbeck SG, Jackson JG, Van Den Berg CL, Weng C, Lee AV. Yee D. Insulin-like growth factor binding protein-3 and insulin receptor substrate-1 in breast cancer: correlation with clinical parameters and disease-free survival. Clin Cancer Res 1997; 3:103-9.

56) Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, Ng L, Simpson DM. Rosenfeld RG. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int J Biochem Cell Biol 1996; 28:619-37.

57) Yen SCC. The biology of the menopause. J Reprod Med 1977; 18:287-96.

58) Chaudhuri PK. Chaudhuri B. Patel N. Modulation of estrogen receptor by insulin and its biologic significance. Arch Surg 1986; 121:1322-5.

59) De Perola G, Cospite MR, Giaculli VA, Giorgino F, Garruti G, Cignarelli M, Giorgino R. Insulin-like growth factor I and Dehydroepiandrosterone sulphate in obese women. Int J Obesity 1993; 17: 481-3

60) Giampietro O, Navalesi R, Buzzigoli G, Boni C, Benzi L Decrease in plasma glucose concentration during storage at –20 C, 1980, 25:1710-1712.

61) Chan AYW, Swaminathan T, Cockram CS Effectiveness of sodium fluoride as a preservative of glucose in blood Clin Chem 1989; 35:315-317

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)*
Glucose (mg/dL)	81.9 (18.9)	78.6 (10.8)*	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)*	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

* One way ANOVA ($P < .05$): differences between cases and controls.

Table 2 Estimated Risks (RR) of Premenopausal Breast Cancer and 95% confidence limits by serum levels of fasting glucose, insulin, insulin-like-growth factor (IGF)-I, and IGF Binding Proteins (IGFBPs).

	I	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)	**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)	**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)	**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)	**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)	**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)	**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)	**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	

I= Reference category

• Adjusted for age, BMI, age at menarche, age at first birth, parity.

Table 3 Estimated Risks (RR) of Postmenopausal Breast Cancer and 95% confidence limits by serum levels of fasting glucose, insulin, insulin-like-growth factor (IGF)-I, and IGF Binding Proteins (IGFBPs).

	I	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)	**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 (mg/dl)	≤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)	**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)	**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)	**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)	**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)	**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)	**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	

I= Reference category

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

Table 4 Estimated risk of Postmenopausal Breast Cancer associated with glucose metabolism and IGF-I factors by strata of BMI

	LOW BMI ≤26			*HIGH BMI >26*			
Tertiles of Variables	I	II	III	I	II	III	P•
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	

• P values for interactive terms of each variable with BMI

Point estimates were adjusted for age and reproductive variables

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 27 Feb 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed list of technical documents. Request the limited distribution statement assigned to the documents listed be changed to "Approved for public release; distribution unlimited." These documents should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

Phylis Rinehart
PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB243021
ADB262474
ADB284009
ADB257455
ADB257446
ADB261351
ADB259684
ADB282142
ADB285141
ADB272522
ADB284022
ADB283904

encl