Effect of a Low-Fat, High-Fiber Diet and Exercise Intervention on Breast Cancer Risk Factors and Tumor Cell Growth & Apoptosis

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Abstract

Background. Lifestyle factors including diet and exercise are thought to be important in the development of breast cancer (BCa) the most common cancer in U.S. women. Estrogen, insulin and insulin-like growth factor-I (IGF-I) have been identified as independent risk factors for the development of BCa. The purpose of the present study was to investigate the effect of a low-fat, high-fiber diet and exercise intervention on these risk factors. We also studied the effect of serum changes on serum-stimulated growth and apoptosis of 3 ER positive BCa cell lines.

Methods. Fasting serum was obtained from postmenopausal women participants at the Pritikin Longevity Center Residential Program where they were placed on a low-fat (10-15% Kcal), high-fiber (>40 gm/d) diet and attended daily exercise classes for 2 weeks. Serum samples were analyzed for estradiol, insulin, IGF-I and IGFBP-1. Serum was also used to stimulate the growth and assess apoptosis in MCF-7, T-47D and ZR-75-1 BCa cell lines.

Results. Estradiol was reduced in the women on HRT (N=16) as well as those not on HRT (N=10). Insulin and IGF-I were reduced in all women while IGFBP-1 was increased. Growth of the BCa cell lines was reduced from 6.6% for the MCF-7 cells to 18.5% for the T-47D cells while apoptosis was increased from 20% in the ZR-75 to 30% in the T-47D cells (N=12). Analysis by TUNEL confirmed apoptosis in T-47D cells after treatment with serum samples.

Conclusions. A very-low-fat, high-fiber diet combined with daily exercise results in major reductions in recognized risk factors for BCa. These *in vivo* changes in serum factors slowed the growth rate and induced apoptosis in BCa cell lines *in vitro.*

Introduction

Breast cancer is the most common cancer in U.S. women with most of the BCa occurring in postmenopausal women. The international variation in BCa incidence, as well as the migration studies, indicate that lifestyle factors play an important role in the development of BCa. The international data show a positive relationship between dietary fat consumption and BCa. Epidemiologic studies report that women in Westernized countries with a high incidence of BCa are at lower risk if they exercise on a regular basis. Estrogens, insulin and IGF-I act as mitogens to stimulate proliferation in normal breast tissue as well as in breast carcinomas and may be major factors in the development of BCa. The importance of estrogen in the development of BCa has been appreciated for many years. Recently attention has focused on insulin and IGF-I acting through the PI3 kinase/Akt pathway and/or the MAP kinase pathway. Studies have demonstrated crosstalk between the IGF-I and E2 receptors enhancing their actions. In a previous study (Nutr. Cancer 38:158,2000) we reported that postmenopausal women attending the Pritikin Program showed a significant reduction in serum insulin and a significant increase in sex hormone-binding globulin which should reduce the level of free estrogen available to stimulate breast tissue. In men attending the Pritikin Program we reported similar responses to insulin and SHBG (Nutr. Cancer 31:127,1998). Furthermore, we reported that men also showed a reduction in IGF-I with a rise in IGFBP-1 that reduced growth and increased apoptosis of prostate tumor cell lines. No change in IGFBP-3 was noted (Cancer Cause Control 13:929,2002).

The purpose of the present study was to investigate the response of these growth factors postmenopausal women attending the Pritikin Program and the impact of the serum changes on the growth of estrogen receptor positive breast cancer cell lines.

Methods

Subjects. The subjects of this study were postmenopausal women attending the Pritikin Longevity Center Residential Program where they were given a low-fat (10-15% Kcal.), high-fiber (>40 gm/d) diet along with one hour of daily supervised exercise, primarily treadmill walking. The diet consisted of natural whole grains, fruits and vegetables with limited amounts (<3.5 oz) of fish, fowl or lean meat and nonfat milk. Fasting blood samples were obtained on days 1 and 13 of the two-week program, allowed to clot, centrifuged, separated and stored at -80 C until used for analyses. The subjects were also weighed at these times.

Serum Analyses. Samples from 26 subjects were thawed and analyzed for estradiol, insulin, IGF-I and IGFBP-1 using ELISA kits from Diagnostic Systems, Inc. Estradiol was analyzed separately for the 16 women on HT compared to the 10 not on HT. For the other analyses the data were analyzed as one group as there were no significant differences between the two groups.

Cell Culture Studies. MCF-7, T-47D and ZR-75-1 breast cancer cell lines were obtained from the UCLA Breast Cancer Research Center and were grown in 75-cm^2 flasks (Falcon Primaria) in RPMI-1640 medium without phenol red, supplemented with 10% FBS, 200IU penicillin, 200mg/ml streptomycin, and 4nM L-glutamine (Omega Scientific, Inc.). The cells were then plated (5 x 10^3 cells/well) in 96-well plates with 10% FBS medium. After 24 hours, fresh medium (10% FBS or 10% human serum from days 1 and 13) was replaced, and the cells were incubated (37°C, 5% CO2) for 48 hours. FBS was used as a control for the growth assay. Cell growth was determined by CellTiter 96AQ Assay (Promega Corporation) and apoptosis by Cell Death Detection ELISA and modified TUNEL (Roche Applied Science). The data are expressed as a percent of the day 1 or pre values. These experiments were conduced on an additional group of 12 postmenopausal women all on HT.

Statistical Analysis. Data were analyzed using InStat Statistical Software, Graphpad Prism with a paired Student's t-test. Data are presented as mean ± standard error with $p < 0.05$ considered significant.

Results

During the 13 day period body weight was reduced from 85±3.3 to 83.2±2.8 Kg. The following table shows the results from the serum analyses. All post values were significantly ($p < 0.05$) different from pre values.

Serum Parameter	Pre	Post	% Change
Estradiol, HT (pg/mL)	232.0±49.7	151.4±34.2	34 %↓
Estradiol, no HT	24.5±4.3	15.4±1.5	37 %↓
Insulin (µU/mL)	13.2±1.51	9.39±1.04	29 %↓
IGF-I (ng/mL)	206.8±15.6	167.9±8.4	19 %↓
IGFBP-1 (ng/mL)	56.7±7.2	74.9±9.1	32 %↑

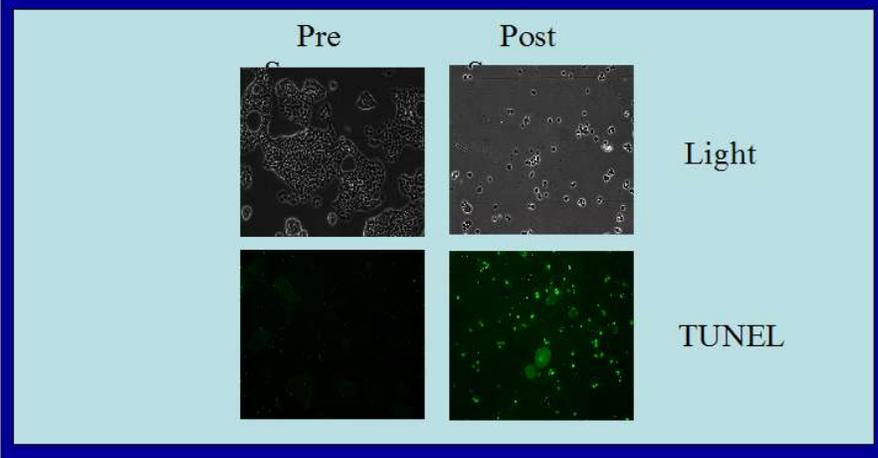**Effect of Diet and Exercise on BCa Cell Growth**
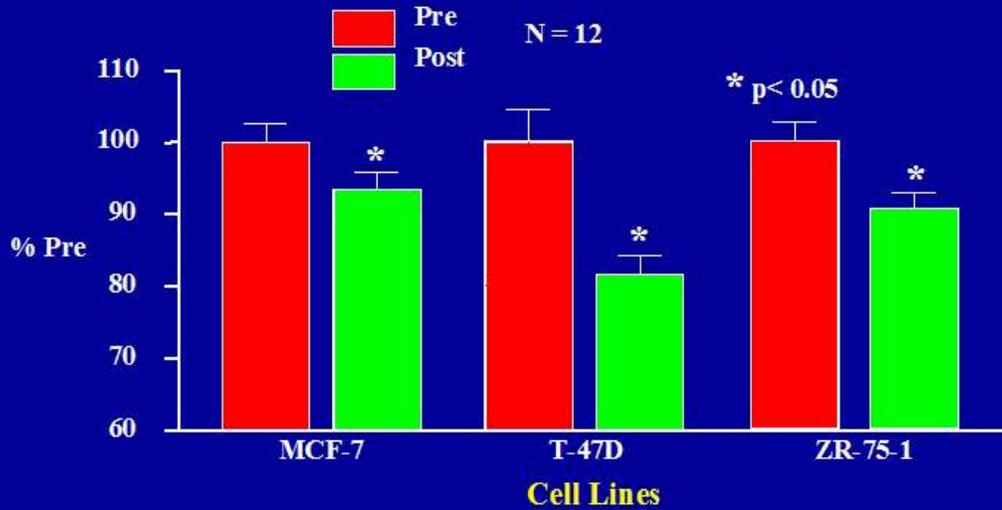
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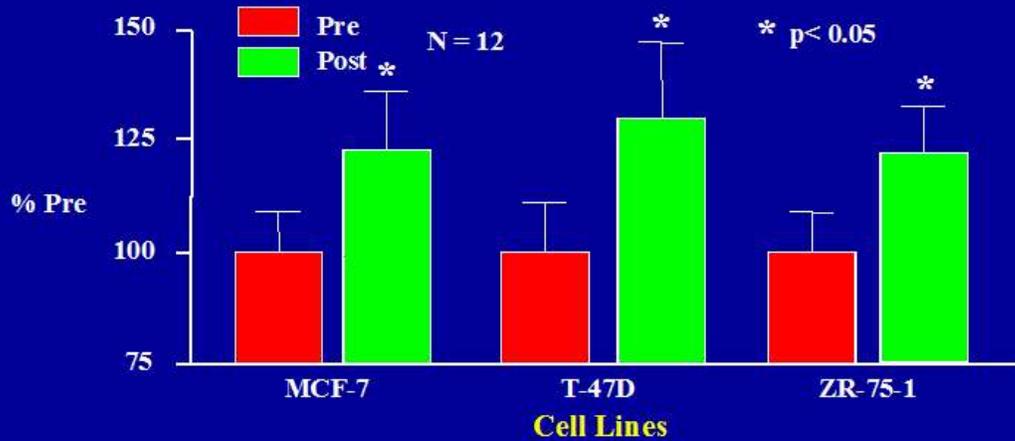
Conclusions

Postmenopausal women adopting a very-low-fat (10-15% Kcal.), high-fiber (>40 gm/d) diet combined with daily exercise have significant reductions in serum estradiol, insulin and IGF-I with significant increases in SHBG and IGFBP-1. The serum changes *in vivo* reduced the growth and induced apoptosis of BCa tumor cell lines *in vitro.* Overall, these results suggest a significant reduction in the risk for BCa.