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Authors

Barnard, R James
Gonzalez, J H
Liva, M E
et al.

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Effects of a Low-Fat, High-Fiber Diet and Exercise Program on Breast Cancer Risk Factors In Vivo and Tumor Cell Growth and Apoptosis In Vitro

R. James Barnard, Jenny Hong Gonzalez, Maud E. Liva, and Tung H. Ngo

Abstract: *The present study investigated the effects of a diet and exercise intervention on known breast cancer (BCa) risk factors, including estrogen, obesity, insulin, and insulin-like growth factor-I (IGF-I), in overweight/obese, postmenopausal women. In addition, using the subjects' pre- and postintervention serum in vitro, serum-stimulated growth and apoptosis of three estrogen receptor–positive BCa cell lines were studied. The women where placed on a low-fat (10–15% kcal), high-fiber (30–40 g per 1,000 kcal/day) diet and attended daily exercise classes for 2 wk. Serum estradiol was reduced in the women on hormone treatment (HT;* n = *28) as well as those not on HT (*n = *10). Serum insulin and IGF-I were significantly reduced in all women, whereas IGF binding protein-1 was increased significantly. In vitro growth of the BCa cell lines was reduced by 6.6% for the MCF-7 cells, 9.9% for the ZR-75-1 cells, and 18.5% for the T-47D cells. Apoptosis was increased by 20% in the ZR-75-1 cells, 23% in the MCF-7 cells, and 30% in the T-47D cells (*n = *12). These results show that a very-low-fat, high-fiber diet combined with daily exercise results in major reductions in risk factors for BCa while subjects remained overweight/obese. These in vivo serum changes slowed the growth and induced apoptosis in serum-stimulated BCa cell lines in vitro.*

Introduction

Breast cancer (BCa) is the most common form of cancer in U.S. women and is the second leading cause of cancer deaths (1). This same situation is found in most developed countries, whereas the incidence of BCa in many developing countries is still quite low (2). The etiology of BCa is poorly understood; however, it is clear that estrogen, either endogenous or exogenous, plays an important role. With menopause the incidence of BCa increases substantially in developed countries but it may actually fall in developing countries (3). Although several genes have been identified as risk factors, it is clear that lifestyle plays an important role (4). When women from countries with a low BCa incidence, such as many Asian countries, migrate to countries such as the United States and adopt a Western lifestyle, the incidence of BCa becomes equal to that found in the host country (5). BCa is also on the rise in Asian countries as they adopt a Western lifestyle (6).

Four lifestyle factors that have received much attention are obesity, diet, physical activity, and alcohol consumption. Obesity is a major risk factor for postmenopausal BCa as reviewed by Harvie et al. (7) and by Rose et al. (8). After menopause, when ovarian production of estrogens ceases, the serum levels of estrogen come from aromatization of androstenedione to estrone in the stroma of fat cells followed by conversion to estradiol (8). The obesity-related increases in circulating estrogens have been associated with increased BCa risk and enhanced progression of estrogen receptor (ER)-positive BCa. In addition to increasing serum estrogens, fat cells release adipokines and cytokines that may be related to BCa (8). Obesity is also associated with insulin resistance and hyperinsulinemia, documented risk factors for BCa (9). Hyperinsulinemia is associated with lower levels of sex hormone–binding globulin (SHBG) and insulin-like growth factor binding protein-1 (IGFBP-1) with elevated levels of IGF-I, all which could increase the risk for BCa (10).

Several aspects of diet have been investigated with conflicting results. According to a recent review by Dumitrescu and Cotarla (11), a high intake of saturated fat and well-done meat is probably associated with increased risk for BCa, whereas a high intake of fruits and vegetables is a well-confirmed negative risk factor. In a review of physical activity and BCa risk, Thune and Furberg (12) reported observations from 41 studies including 108,031 BCa cases. In 26 of 41 studies, increased occupational or leisure time physical activity was associated with approximately a 30% decrease in BCa risk for pre-, peri-, and postmenopausal women. A graded dose response was observed in 16 of 28 studies. In the review by Dumitrescu and Cotarla (11), it was concluded that alcohol intake of approximately one drink per day was associated with an increase in BCa risk.

The purpose of this study was to investigate the effects of lifestyle change, including adopting a low-fat, high-fiber diet

All authors are affiliated with the Department of Physiological Science, University of California, Los Angeles, Los Angeles, CA 90095–1606.

and exercise program along with alcohol abstinence, on known serum BCa risk factors in overweight/obese, postmenopausal women. The risk factors included estradiol, insulin, and IGF-I. In addition, we tested the effects of serum changes on serum-stimulated growth and apoptosis of three ER-positive BCa cell lines. The results showed major reductions in serum risk factors in vivo as well as reduced growth and increased apoptosis in the serum-stimulated ER-positive BCa cell lines in vitro.

Materials and Methods

Subjects and Intervention

The Human Subjects Protection Committee at UCLA approved the research protocol, and all volunteers gave informed consent to participate in the study. The subjects were 38 overweight/obese, postmenopausal women, ages 51–79, who attended the Pritikin Longevity Center 14-day Residential Program. The women were generally in good health with no problems that would prohibit them from participating in the diet and exercise intervention. The study consisted of two groups of women. Group 1 consisted of 26 women (16 on hormone treatment, HT, and 10 not on HT). This group participated in an earlier study (13), and their serum was subsequently used to measure estradiol, insulin, IGF-I, and IGFBP-1. To do the cell culture studies, a second group of 12 volunteers was recruited (group 2), all on HT. Serum estradiol and IGF-I were measured in this group, in addition to conducting the in vitro, serum-stimulated cell culture studies. Fasting blood samples were obtained on Days 1 and 13 between 6:30 and 8:00 am and were centrifuged to separate the serum that was stored at –80°C until used for the assays.

During the intervention, subjects were given prepared meals that contained 10–15% calories from fat (polyunsaturated/saturated fatty acid ratio = 1.24). Fifteen to 20% of the calories were from protein, primarily from a plant source, two servings of nonfat milk, and no more than 3.5 oz of fish or fowl. The remaining calories, 70–75%, were obtained from carbohydrates in the form of vegetables, fruits, legumes, and whole grains. Dietary fiber (30–40 g/1,000 kcal each day) was consumed. Finally, less than 100 mg of cholesterol and 4 g of sodium chloride per day were consumed. No alcohol, tobacco, or caffeine consumption was permitted. The food was provided ad libitum except for the animal protein, which was restricted to ≤3.5 oz/day. The subjects readily consumed the food, the only major complaint being the flatulence initially associated with adopting this type of diet.

All subjects received a history and physical exam by a physician and were given a treadmill stress test prior to starting the exercise program. During the 2-wk program, the women exercised at their assigned training heart rate for 30–60 min a day 4–5 days a week. On 1–2 days a week, the subjects exercised below their training heart rate for 40–60 min. The exercise was supervised and consisted of treadmill walking or using other aerobic exercise devices.

Serum Assays

Fasting glucose data were obtained from the medical charts and were part of the standard medical workup. Estradiol, insulin, IGF-I, and IGFBP-1 were all determined in duplicate by enzyme-linked immunosorbent assay (ELISA) with kits from Diagnostic Systems Laboratories (Webster, TX). The glucose and insulin data were used to calculate insulin resistance via the homeostasis model assessment (HOMA-IR) calculated as [fasting insulin (μU/ml) × fasting glucose (mmol/l)]/22.5 (14).

Cell Culture

Three ER-positive BCa cell lines (MCF-7, T-47D, and ZR-75-1) were obtained from the UCLA Cancer Center. These are three commonly used BCa cell lines due to their estrogen responsiveness. The cells were grown in 75-cm^2 flasks (Falcon) in RPMI-1640 medium without phenol red and supplemented with 10% fetal bovine serum (FBS), 200 IU penicillin, 200 mg/ml streptomycin, and 4 nM l-glutamine (all from Omega Scientific, Tarzana, CA). The cells were maintained at 37°C and supplemented with 5% CO_2 in a humidified incubator. Cells were passaged when they reached 80% confluence, and media was changed every 3 days. The passage number of cells used in the experiments did not exceed 10. At 80% confluence, cells were detached by first washing with phosphate-buffered saline (PBS) without Ca^{2+} or Mg^{2+} salts (Omega Scientific) and then incubated with 1 ml of 0.25% trypsin-EDTA solution (Sigma Chemical Co., St. Louis, MO) and 10 ml of cell media added to the flask to stop the trypsin. The cells were removed from the flask and centrifuged at 357 *g* and then resuspended in fresh medium with FBS. Cell viability was assessed by 0.4% trypan blue solution exclusion (Sigma Chemical Co.).

Cell Growth

Cell growth was determined by the CellTiter $96AQ_{ueous}$ One Solution Cell Proliferation Assay (Promega, Madison, WI). Cells were plated at a density of 5,000 per well in 96-well tissue culture plates. After the initial 24-h incubation, total media was removed from the wells, and 90 μl of fresh media was added to each well with 10 μl of FBS or subject serum. The plates were incubated (37°C, 5% CO_2) for 48 h. Following treatment with the CellTiter solution, optical density was measured at 490 nm in a plate reader (Molecular Devices, Sunnyvale, CA). Pre- and postintervention sera were incubated on the same plate, and the results were expressed as a percent of the absorbance found in the presamples. All tests were run in triplicate.

Apoptosis

Cell Death Detection ELISA^{plus} (Roche, Indianapolis, IN) was used to detect apoptosis in the BCa cells. The cells were plated at a density of 10,000 per well in 96-well plates with 10% FBS media. After 24 h, fresh media (10% FBS or 10% human serum, pre- and postintervention) was replaced, and the cells were incubated (37°C, 5% CO_2) for 48 h as done for the growth assays. The plates were read at 405 nm with all tests run in triplicate.

To confirm apoptosis, the In Situ TUNEL (Roche) reaction was conducted on three subjects, pre- and postintervention. T-47D cells were plated at a density of 80,000 cells per well in 8-well plates (Nalge Nunc, Lab-Tek Chamber Slide, Napersville, IL) with 10% FBS media. After 24 h, fresh media (10% FBS or 10% human pre- and postintervention) was replaced, and the cells were incubated (37°C, 5% CO_2) for 48 h. Media was removed, and fresh 4% paraformaldehyde in PBS (pH 7.4) was added to each well for 1 h at 15–25°C to fix the BCa cells. Wells were rinsed two times with PBS and incubated for 2 min on ice with 0.1% Triton X-100 in 0.1% sodium citrate to permeabilize the cells. Wells were rinsed twice with PBS and air dried before adding 50 µl of TUNEL reaction mixture and covered with parafilm before incubating at 37°C for 60 min. Cells were rinsed three times with PBS, and samples were immediately analyzed using an inverted fluorescence microscope. An excitation wavelength in the range of 515–565 nm was used. All samples were done in duplicate.

Statistical Analysis

Statistical analyses were performed between pre- and postintervention data by paired Student's *t*-test using Instat Statistical Software (Graph-pad, San Diego, CA). Data are expressed as mean ± standard error and $P < 0.05$ was considered significant.

Results

Data were collected from 38 overweight/obese, postmenopausal women who voluntarily participated in the 2-wk diet and exercise intervention. For the women in group 1 ($n = 26$), weight was reduced from 85.0 ± 3.3 to 83.2 ± 2.8 kg, and body mass index (BMI) was reduced from 32.4 ± 1.5 to 30.9 ± 1.2 kg/m^2; thus, the women were still overweight/obese at the end of the 2-wk intervention. Fasting glucose was reduced from 6.84 ± 2.1 to 6.08 ± 1.0 mmol/l; $P < 0.01$. Table 1 shows that insulin was also reduced. Using these two pieces of data, insulin resistance was calculated as HOMA-IR and was reduced from 4.7 ± 0.4 to 2.9 ± 0.2; $P < 0.01$. Estradiol was reduced in both the women on HT and those not on HT (Table 1). As there were no significant differences between the two HT subgroups for the other risk factors, that is, insulin, IGF-I, and IGFBP-1, the data were analyzed as one group. As can be seen in Table 1, IGF-I was significantly reduced and IGFBP-1 was significantly increased. The increase in IGFBP-1 along with the decrease in IGF-I would indicate a reduction in the amount of free IGF-I.

The women in group 2 were very similar to the women in group 1 except that all 12 were on HT. Their weight was reduced from 81.4 ± 12.3 to 78.9 ± 11.1 kg, and BMI was reduced from 30.2 ± 2.1 to 29.4 ± 1.9 kg/m^2. To assure that the women in group 2 were responding to the intervention as the women in group 1 did, we measured serum estradiol and IGF-I. Estradiol was reduced from 199.6 ± 29.0 to 122.1 ± 12.54 pg/ml, whereas IGF-I was reduced from 184.1 ± 19.6 to 162.3 ± 20.0 ng/ml; both $P < 0.05$. Figure 1 shows the results from the serum-stimulated cell culture studies. Growth over 2 days of stimulation was reduced by 6.6 ± 2.2% for the MCF-7 cells, by 9.9 ± 2.1% for the ZR-75-1 cells, and by 18.5 ± 2.8% for the T-47D cells. With the Cell Death ELISA assay, apoptosis was increased by 23 ± 13% for the MCF-7 cells, by 20 ± 11% for the ZR-75-1 cells, and by 30 ± 17.5% for the T-47D cells (Fig. 2). To confirm the apoptosis, TUNEL staining was done on some of the T-47D cells with pre- and postintervention serum. Figure 3 shows results for both the light microscope and TUNEL staining. For the light microscope, the cells grown in the postintervention serum appear quite different from the cells grown in the preintervention serum. The TUNEL staining showed almost no apoptosis with the preintervention serum stimulation but a significant amount of apoptosis in the postintervention serum-stimulated samples. In fact, there appeared to be more apoptosis than what was quantified with the Cell Death ELISA. These TUNEL results were consistent in all three subjects.

Table 1. Effect of Diet and Exercise Intervention on Serum BCa Risk Factors[a]

Serum Parameter	Preintervention	Postintervention	% 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 ± 1.5	9.4 ± 1.0	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 ↓

a: Abbreviations are as follows: BCa, breast cancer; HT, hormone treatment; IGF-I, insulin-like growth factor-I; IGFBP-1, IGF binding protein-1. $n = 26$, 16 on HT and 10 with no HT. Values are mean ± SE; all changes significant $P < 0.05$.

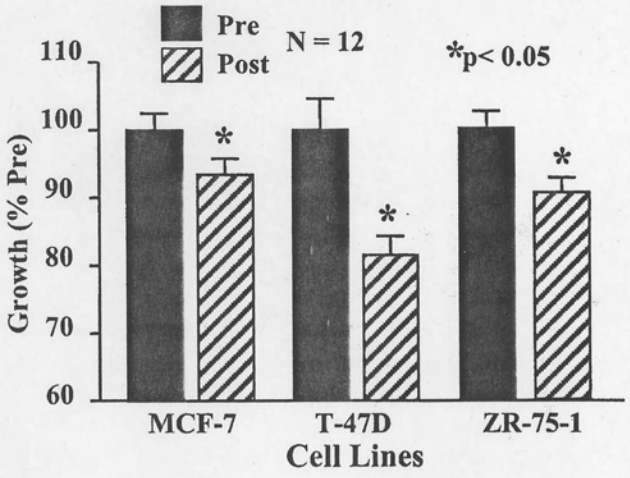

Figure 1. Effects of diet and exercise intervention on growth of breast cancer (BCa) cell lines. BCa cells were plated overnight in 10% fetal bovine serum, and the following day the media was removed and replaced with fresh media and 10% human serum pre- and postintervention. The cells were allowed to grow for 2 days, and growth was determined by the CellTiter Proliferation Assay (Promega, Madison, WI).

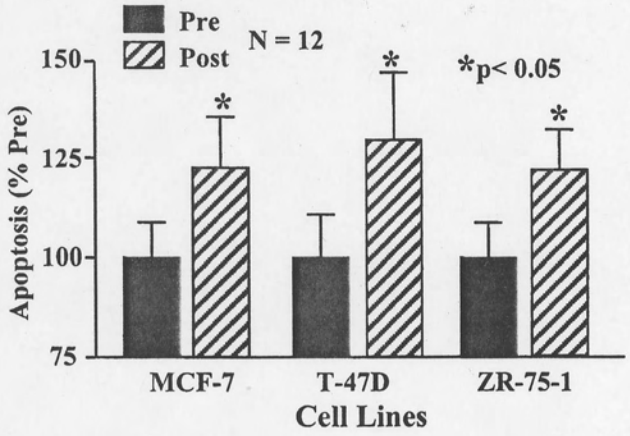

Figure 2. Effects of diet and exercise intervention on apoptosis in breast cancer (BCa) cell lines. BCa cells were plated overnight in 10% fetal bovine serum, and the following day the media was removed and replaced with fresh media and 10% human serum pre- and postintervention. The cells were allowed to grow for 2 days, and apoptosis was determined by the Cell Death Detection enzyme-linked immunosorbent assay (Roche, Indianapolis, IN).

Discussion

The results of this study show that, when overweight/obese, postmenopausal women adopt a very-low-fat, high-fiber diet consisting mainly of fruits, vegetables, and whole grains with limited amounts of animal protein, and do daily aerobic exercise, major reductions in BCa risk factors can be achieved in 2 wk. Reductions in serum estradiol, insulin, and IGF-I were observed while the subjects remained overweight/obese. The reductions in these risk factors were major, ranging from 19% for IGF-I to as high as 37% for estradiol in women not on HT. These reductions should be clinically relevant. Furthermore, these in vivo serum changes reduced growth and induced apoptosis in three ER-positive BCa cell lines in vitro. These observed changes should translate into a major reduction in the risk for BCa, especially if confirmed in larger studies.

Obesity has been consistently shown to be a major risk factor for BCa in postmenopausal women but appears to be protective in premenopausal women (8,15). In postmenopausal women, fat tissue becomes the major site of estrogen production through aromatization of androgens. Regardless of menopausal status, obese women are more likely to have metastatic BCa at the time of diagnosis (15). In the present study, the postmenopausal women lost only a small amount of weight and remained obese or overweight at the end of the 2-wk intervention. However, estradiol was reduced by 35–40%, indicating that something associated with obesity was possibly stimulating aromatase activity in the fat tissue. Keinan-Boker et al. (16) have suggested that both insulin and IGF-I might stimulate sex steroid synthesis in addition to their well-known effect of inhibiting the synthesis of sex steroid–binding proteins. McTernan et al. (17) reported an increase in aromatase activity following insulin stimulation of abdominal adipose tissue removed from either pre- or postmenopausal women. As both insulin and IGF-I were reduced in our subjects, this might account for the reduction in estradiol. Another possibility is that the high-fiber content of the diet increased estrogen excretion via the biliary system, resulting in less reabsorption into the circulation (18). A meta-analysis of 12 case-control studies by Howe et al. (19) showed a risk ratio of 0.85 for women consuming 20 g/day of dietary fiber. In the present study, the women consumed 30–40 g of dietary fiber per 1,000 kcal/day.

The reduction in estradiol observed in this study agrees with an earlier study with the same intervention where a 50% reduction was reported (20). Others have also reported that adopting a low-fat, high-fiber diet lowers serum estrogens in both pre- and postmenopausal women (21–24). The reduction in estradiol observed in this study was evident in both women on HT and those not on HT. The reduced estradiol in the women on HT may be very significant as several studies have documented the increased risk for BCa in women on HT (25). Prior to the recent randomized trials, HT was highly recommended for the prevention of heart disease and osteoporosis. However, the randomized trials showed not only an increase in BCa but also an increase in cardiovascular events (26). It should be noted that the women in group 1 of the present study also had major reductions in several cardiovascular risk factors as previously reported (13).

The role of dietary fat in BCa has been controversial. Numerous rodent studies have shown that high-fat diets increase BCa development substantially (27). For humans, a strong positive correlation has been reported for estimates of per capita fat consumption and BCa incidence/mortality (28). It has been suggested that part of the response in countries consuming a low-fat diet might be due also to a higher reproductive rate, known to reduce the risk for BCa. Studies within a given

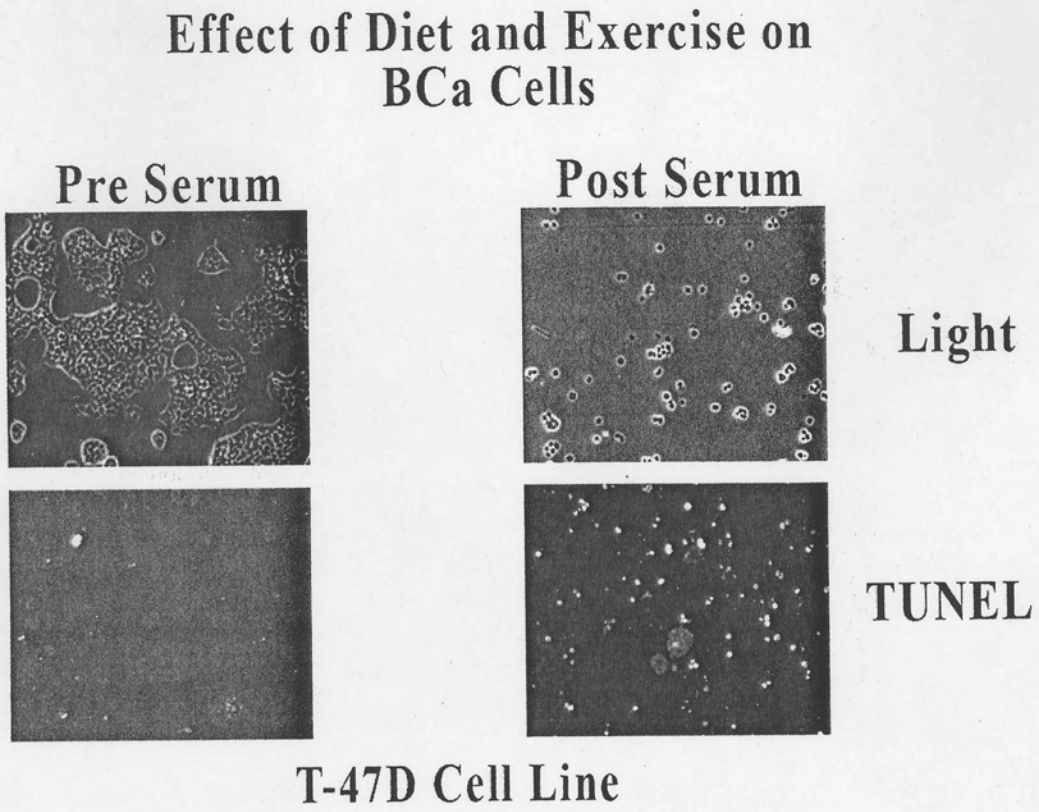

Figure 3. Effects of diet and exercise intervention on apoptosis determined by TUNEL. To confirm apoptosis in the T-47D cell line, the cells were grown for 2 days as in Figs. 1 and 2 and then fixed for the TUNEL assay and fluorescent microscopy.

population, including several in the United States, have been inconsistent. Following a pooled data analysis of 12 case-control studies, Howe et al. (19) found a statistically significant relation between dietary saturated fat and BCa in postmenopausal women. In another pooled analysis of data in 1996, Hunter et al. (29) stated, "In the context of a Western lifestyle, lowering the total intake of fat in midlife is unlikely to reduce the risk of BCa substantially." The reduction in risk factors observed in the present study would suggest the opposite conclusion. The lack of a correlation between dietary fat and BCa is puzzling because dietary fat has been correlated with obesity, a major risk factor for postmenopausal BCa. As well as reducing serum estrogen, the present diet and exercise intervention has been shown to increase the serum level of SHBG, which should further reduce the level of free estrogen available to interact with ERs (30).

In addition to being correlated with BCa in postmenopausal women, obesity is correlated with insulin resistance, hyperinsulinemia, and type 2 diabetes. Thus, it is not surprising to find that type 2 diabetes is associated with a 10–20% excess relative risk for BCa (9). Others (10,16,31,32) have reported that elevated C-protein or fasting insulin is associated with increased BCa risk. In the present study, serum insulin and calculated insulin resistance (HOMA-IR) were both substantially reduced in the women in group 1. These changes are likely due in large part to the daily exercise as exercise has an insulin-like effect and is known to reduce insulin resistance (33). Insulin is a well-recognized mitogen and has been shown to stimulate the growth of BCa cell lines (34).

Insulin, in addition to being a mitogen, is known to have an impact on the liver to reduce production of SHBG and IGFBP-1 while stimulating production of IGF-I (35,36). In the present study, serum insulin and IGF-I were significantly reduced, whereas IGFBP-1 was increased in the women in group 1. The increase in IGFBP-1 should further reduce the level of free IGF-I. IGF-I is also a known mitogen and has been shown to block apoptosis in several different cell types (37). Epidemiological data have found elevated serum IGF-I to be a BCa risk factor in premenopausal women in five of eight studies; however, the data for postmenopausal women have been mixed, which is surprising (38). The reduction in both estradiol and IGF-I in the present study is especially important as there is cross talk between the estrogen and IGF-I receptors in BCa cells at both the genomic and nongenomic levels (39,40). In addition to the interaction between estrogen and IGF-I, it is thought that insulin and IGF-I act synergistically to increase BCa risk (10). They also act synergistically to stimulate motility in BCa cell lines, an effect that could enhance migration and invasion (41). IGFBP-1 has been shown to block this synergistic effect on motility (42). Thus, the reduction in all three of these factors as well as the increase in IGFBP-1 in the present study should represent an important reduction in the risk for BCa. These changes should also re-

duce the risk for recurrence of BCa in agreement with epidemiological studies showing the value of a low-fat diet or regular exercise (43,44).

To further explore the effects of the serum changes, we used three ER-positive BCa cell lines and subjected them to serum stimulation using the pre- and postintervention serum. With the preintervention serum, all three cell lines showed robust growth with little apoptosis. With the postintervention serum, cell growth was reduced and apoptosis increased in all three of the cell lines. In previous studies with males, using androgen-dependent prostate cancer (PCa) cell lines, we showed similar, but more dramatic, responses to serum stimulation postintervention (45). The preintervention serum-stimulated PCa cells also showed robust growth with little apoptosis, similar to that observed with the BCa cell lines. Thus, it is not surprising that the international data show a strong correlation between PCa and BCa and that these are the two most common cancers in the United States. Alterations in the IGF axis with the diet and exercise intervention appeared to be the major factors in the PCa studies as adding IGF-I back to the postintervention serum eliminated the observed reduction in serum-stimulated PCa cell growth. Arteaga and Osborne (46) reported that blocking the IGF-I receptor reduced growth in six of seven BCa cell lines, including the MCF-7 and ZR-75-1 lines used in the present study. These two cell lines have also been reported to have insulin receptors and may experience insulin-stimulated growth (47). The increase in IGFBP-1 in the present study may also be important because it has been shown to inhibit motility in BCa cells (42).

Intervention trials with various dietary supplements for cancer prevention have provided mixed results with some even reporting an increase in cancer incidence (48). Instead of looking for the magic bullet with individual or combined supplemental nutrients and/or phytochemicals, a total lifestyle change consisting of a low-fat, high-fiber diet of natural foods including fruits, vegetables, and whole grains with limited amounts of animal protein combined with daily exercise (45–60 min) may be more efficacious. Rock et al. (49) recently reported the results of a dietary intervention study in women with BCa where consumption of fruits, vegetables, and fiber was increased and fat was reduced. After 1 yr, bioavailable serum estradiol was reduced, and fiber intake was independently related to reductions in serum estradiol. This type of diet is high in antioxidants and phytochemicals, which may be important in preventing the initiation of cancer. The importance of daily exercise should not be underestimated. In our PCa studies, we have shown that daily exercise alone enhances the cellular level of p53, a gene known to inhibit cell division and induce cell repair or apoptosis (50). This suggested diet and exercise lifestyle modification is consistent with the recent epidemiological data reported by Malin et al. (51) showing that a lack of physical activity combined with a high BMI/high energy intake resulted in a major increased risk for BCa. The association was stronger in postmenopausal than premenopausal women. Postmenopausal women who were not exercising and had a high BMI had an odds ratio of 4.74 for BCa.

Limitations to the present study include the fact that it was not a randomized controlled study and was short term with the women in obvious negative energy balance. However, in our PCa studies, we reported that long-term compliance (14 yr) to the low-fat diet and exercise program resulted in greater changes in the IGF axis and greater impact on PCa tumor cell growth and apoptosis (45). The results obtained in this study only pertain to overweight/obese women. Larger studies, including normal-weight women, are needed.

In conclusion, a lifestyle change consisting of a low-fat (10–15% calories), high-fiber (30–40 g/1,000 kcal/day) diet from natural foods (fruits, vegetables, and whole grains) combined with 60 min of daily aerobic exercise significantly reduces serum BCa risk factors including estradiol, insulin, and IGF-I in overweight/obese, postmenopausal women. Serum IGFBP-1 was increased, which should further reduce the amount of free IGF-I. These changes were found in both women on HT and those not on HT. The in vivo serum changes reduced growth and induced apoptosis with in vitro serum stimulation in three different ER-positive BCa cell lines. The results suggest a significant reduction in the risk for BCa or for BCa recurrence.

Acknowledgments and Notes

This study was supported by funds from UCLA and a donation from the L.B. Research and Education Foundation. Address correspondence to R. J. Barnard, Department of Physiological Science, UCLA, 621 Charles E. Young Dr. So., Los Angeles, CA 90095–1601. Phone: 310–825–3794. FAX: 310–206–9184. E-mail: jbarnard@physci.ucla.edu.

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