Journal of Cellular Biochemistry Supplements 28/29:111–116 (1997)

Dose-Ranging Study of Indole-3-Carbinol for Breast Cancer Prevention

George Y.C. Wong,* Leon Bradlow, Daniel Sepkovic, Stephanie Mehl, Joshua Mailman, and Michael P. Osborne

Strang Cancer Prevention Center, New York, New York

Abstract Sixty women at increased risk for breast cancer were enrolled in a placebo-controlled, double-blind dose-ranging chemoprevention study of indole-3-carbinol (I3C). Fifty-seven of these women with a mean age of 47 years (range 22–74) completed the study. Each woman took a placebo capsule or an I3C capsule daily for a total of 4 weeks; none of the women experienced any significant toxicity effects. The urinary estrogen metabolite ratio of 2-hydroxyestrone to 16α-hydroxyestrone, as determined by an ELISA assay, served as the surrogate endpoint biomarker (SEB). Perturbation in the levels of SEB from baseline was comparable among women in the control (C) group and the 50, 100, and 200 mg low-dose (LD) group. Similarly, it was comparable among women in the 300 and 400 mg high-dose (HD) group. Regression analysis showed that peak relative change of SEB for women in the HD group was significantly greater than that for women in the C and LD groups by an amount that was inversely related to baseline ratio; the difference at the median baseline ratio was 0.48 with 95 % confidence interval (0.30, 0.67). No other factors, such as age and menopausal status, were found to be significant in the regression analysis. The results in this study suggest that I3C at a minimum effective dose schedule of 300 mg per day is a promising chemopreventive agent for breast cancer prevention. A larger study to validate these results and to identify an optimal effective dose schedule of I3C for long-term breast cancer chemoprevention will be necessary. J. Cell. Biochem. Suppls. 28/29:111–116.

Key words: chemoprevention; estrogen metabolites; surrogate endpoint biomarker

Indole-3-carbinol (I3C) is a compound present in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, and cauliflower. This compound has been shown to protect against certain chemical carcinogens, and to induce the enzyme P450A1, which is responsible for the formation of the estrogen metabolite 2-hydroxyestrone [1]. Cell culture experiments have shown that 2-hydroxyestrone acts to block proliferation and inhibit promotion of anchorage independent growth in mouse mammary cells, while its competitive counterpart 16α-hydroxyestrone acts in a promotional manner [2,3]. Therefore, the ratio of 2-hydroxyestrone to 16α-hydroxyestrone, as determined by an ELISA assay [4], is a potential surrogate endpoint biomarker (SEB) for breast cancer prevention. Two animal studies have shown that elevating the estrogen metabolite ratio protects against mammary tumor formation. Bradlow et al. [5] showed this to be the case in the C3HOuJ model, and Grubbs et al. [6] showed this in the DMBA-induced rat model. In the latter case, protection was almost complete. A study in women at various levels of breast cancer risk showed that 16α-hydroxyestrone was elevated in women at greater familial risk for breast cancer [7]. The same phenomenon had been observed in mice at different levels of breast cancer risk [8]. In a recent study, women who had a low metabolite ratio due primarily to the presence of an enzyme defect, which blocks 2-hydroxylation of estradiol, showed a 10-fold increase in breast cancer incidence [9]. The ability of I3C to promote 2-hydroxylation has been demonstrated both in breast cancer cell culture experiments [10,11] and in animal studies [5,6].

The ability of I3C to induce a significant increase in 2-hydroxylation in humans in a short time was first demonstrated by Michnovicz and Bradlow [12]. A 3-month trial of I3C at 400 mg per day against a placebo control and a high fiber diet control showed that the metabo-

Contract grant sponsor: Murray and Isabella Rayburn Foundation; Contract grant sponsor: Tiger Foundation.

*Correspondence to: George Y.C. Wong, Director of Preventive Oncology Research, Strang Cancer Prevention Center, 428 East 72nd Street, New York, NY 10021.

Received 29 November 1996; Accepted 12 November 1997

lite shift in favor of 2-hydroxylation pathway was sustained over the entire trial period and that no significant adverse effects were observed [13]. The results from these studies suggest that I3C may be a promising chemopreventive agent for breast cancer prevention. We launched a short-term dose-ranging study of I3C in women at increased risk for breast cancer. The overall aim of the intervention study was to determine a minimum effective dose (MED) of I3C, which will not exceed the safely tolerated dose of 400 mg per day established [13] and which will result in a sustained increase in 2-hydroxylation over a 4-week trial period. Five doses of I3C were considered: 50, 100, 200, 300, and 400 mg. A secondary objective of the study was to assess toxicity effects of I3C when taken daily for 4 consecutive weeks. The SEB used in this study was ratio of urinary 2-hydroxyestrone to 16α-hydroxyestrone. Sixty women were recruited in the study, and full compliance was obtained in 57. A placebo-controlled, double-blind trial design was adopted for the study. MED was statistically determined to be 300 mg, and a significant difference was established in the up-regulation of the SEB between the MED group and the placebo group. No significant toxicity effects were observed in the 57 women at the end of the 4-week trial.

STUDY POPULATION

Adult women in good general health but at increased risk for breast cancer were candidates for the dose-ranging study. A woman is considered to be at increased risk for breast cancer either if she is over 60 years of age or she has a family history of the disease (at least one first-degree relative or at least two second-degree relatives with a history of breast cancer). Women who have had a diagnosis of lobular carcinoma in situ or atypia hyperplasia are also considered to be at increased risk in our study.

A number of exclusion criteria were imposed in order to minimize the chances of confounding the outcome of the particular estrogen biomarker chosen. These included thyroid disorders, regular cigarette smoking within the last 6 months, obesity defined as 25% overweight using the nomograph for Body Mass Index, severe anorexia, breast feeding, pregnancy or intention to become pregnant during the study period. In addition, women who have had any form of cancer other than basal or squamous cell carcinoma of the skin, or carcinoma in situ of the cervix, were excluded from the study. Finally, women who regularly consume a large amount of cruciferous vegetables were also excluded because of the nature of our intervention study.

A total of 60 women who were eligible for the trial were selected from over 100 women who were eager to participate in the study. Most of the women came from the New York metropolitan area. Each eligible woman was required to sign an informed consent form before entering the study.

STUDY DESIGN

A placebo-controlled, double-blind design was adopted for the dose-ranging study. Because a rigorous toxicity analysis had not been previously carried out, a dose-escalation scheme was used in the dose assignment for safety considerations. First, ten women in the control group were given placebo capsules. This was followed by assignments of ten women to each of the five ascending dose groups.

A pre-menopausal participant was asked to schedule her appointment within 3 days after her next period ended. Every participant was asked to bring in two first morning urine samples, one from the morning prior to the appointment and the other from the morning of the appointment. A blood sample was taken from each eligible woman on the appointment day and she was given a bottle containing seven capsules of placebo or I3C. One week later, for a total of 4 weeks, a first morning urine sample and a blood sample were collected, and a refill was dispensed for the following week. Because no reliable biochemical tests for I3C metabolites are available, compliance monitoring was carried out by both pill count and an interview.

STATISTICAL METHODS

In the dose-ranging analysis, the level of perturbation of the SEB, namely the urinary estrogen ratio, at any time point was expressed as relative change from baseline. For each dose group, including the placebo group, the peak relative change (PRC) over the 4-week trial period was obtained for each woman, and the mean of the PRC was used to estimate the peak relative perturbation for the particular dose group over the trial period. We remark that a more sensitive approach utilizing a parametric statistical model was not feasible here because

the individual response profiles could not be summarized by a simple parametric curve (for instance, a sigmoidal curve). The estimated PRC from each dose group was then plotted against a dose of I3C to search for an MED. Our dose-ranging study suggests a clear dichotomy of response between a low-dose group involving 50, 100, and 200 mg, and a high-dose group involving 300 and 400 mg; therefore, parametric model fitting at this stage of dose-ranging study to identify an MED was not necessary. Comparisons of PRC among the dose groups were adjusted for confounding factors using linear regression. To ensure no serious statistical biases were introduced into the dose-ranging analysis due to non-randomness of dose assignment, distributions of various factors that could contribute to biases were compared across the three dose groups.

FOOD ITEM ANALYSIS

Every participant was required to complete a simple food intake questionnaire regarding her eating habits in the past 3 months preceding her initial interview for the intervention trial. Both the frequency and the serving size of a variety of vegetables, including most known I3C-rich vegetables, were recorded for each woman. A numeric score representing the total monthly consumption of a specific vegetable item was calculated from the food intake data. Assuming equal weight for every vegetable item, we derived for each woman a I3C vegetable consumption score and the proportion of I3C vegetables in the total vegetables consumed averaging over a month. Data from a total of 54 participants were available for such a food intake analysis. Both the I3C score and the proportion of I3C vegetable consumption were not significantly related to baseline urinary estrogen ratio.

TOXICITY ANALYSIS

Clinical chemistry and complete blood counts were determined from the blood samples collected at baseline and at the end of each of the 4 consecutive weeks of trial. Any parameter whose measured value was outside the normal range was investigated for possible toxicity. Except for two participants who had unexplained small increases in the liver enzyme SGPT level (43 to 65, and 30 to 71), no other toxicity effects were encountered.

DOSE-RANGING ANALYSIS

A total of 57 women were evaluable for the entire dose-ranging study. Except for three women from New Jersey, all of the 57 women were from the New York metropolitan area. Fifty-two (91%) of the women were white. Forty-six (81%) were college educated, and twenty-four (42%) completed graduate studies. The average age of the participants was 46.7 years (range 22–74). The average age at menarche was 12.4 years (range 8–18). Forty (70%) of the women were pre-menopausal, and 38 (67%) of the women have been pregnant at least once.

Figure 1 displays the sample mean relative change of the estrogen ratio from baseline over time for the control group (n = 10), 50 mg group (n = 7), 100 mg group (n = 10), 200 mg group (n = 10), 300 mg group (n = 10), and 400 mg group (n = 10). The profiles suggest a segregation of the treated groups into a low-dose group (LD) consisting of women in the 50, 100, and 200 mg groups, and a high-dose (HD) group consisting of women in the 300 and 400 mg groups. Moreover, the plots also suggest that the control group (C) was not significantly different from the LD group. For the sake of statistical power, the dose-ranging analysis hereafter will compare data from the C, LD, and HD groups.

Before we can statistically compare the levels of perturbation of the SEB in the three groups, we have to rule out the presence of any statistical bias due to non-randomness of dose assignments to the participants. To this end, we examined the distributions of a number of potential

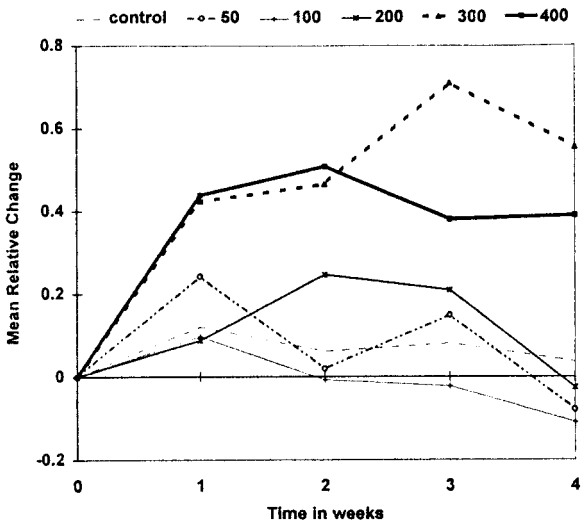

Fig. 1. Mean relative change of urinary estrogen ratio profile plots for the control and five dose groups.

confounding factors, including age, age at menarche, baseline estrogen ratio, menopausal status, pregnancy history, and educational level. No significant differences were found across the three groups with respect to such factors.

Within each of the three groups, we identified the PRC for each of the participants in the group and calculated the usual 95% confidence interval (CI) for the population mean PRC for the group. Figure 2 presents the individual PRC values and the CI for each group. There was no significant difference in mean PRC between C and LD. The sample mean ± SD of PRC for LD was 0.33 ± 0.36 and that for HD was 0.81 ± 0.57; the difference of 0.48 was significant at $P = 0.001$ by the two-sample *t*-test. The 95% CI for the difference in mean PRC between the HD and LD groups was estimated to be (0.22, 0.76).

The perturbation results were unadjusted for any confounding factors. Menopausal status was a major concern in the comparison. Figure 3a,b shows that within each of HD group and C + LD group, there was no significant difference in mean relative change of the SEB from baseline between pre-menopausal and post-menopausal women over the entire trial period. The same conclusion was true for comparison based on PRC. Besides menopausal status, we also included age, age at menarche, baseline estrogen ratio, and educational level in a multivariate

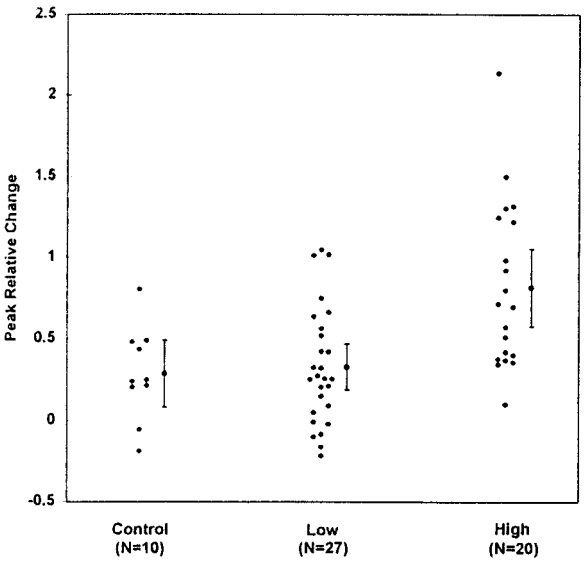

Fig. 2. Comparison of peak relative change of urinary estrogen ratio among control, low- and high-dose groups. Difference between the high-dose group and the other two dose groups, unadjusted for confounding factors, was significant at $P = 0.05$.

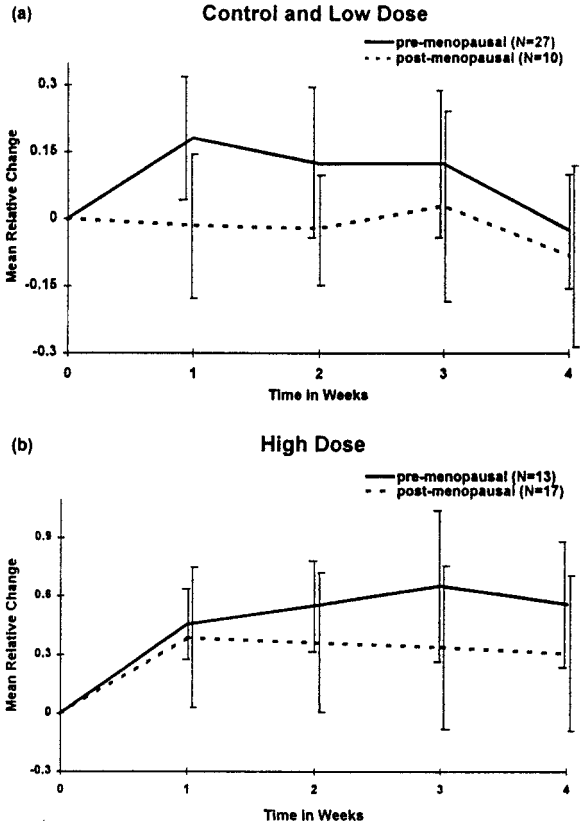

Fig. 3. a,b: Mean relative change of urinary estrogen ratio profile plots stratified by menopausal status. Vertical bars represent usual 95% confidence intervals for the mean. No significant difference in mean relative change between pre-menopausal and post-menopausal women was established within both control + low-dose group and high-dose group.

regression to attempt to explain the variation in the observed PRC. For the C + LD group, the variation in PRC could only be explained by random inter-participant differences. However, for the HD group, about 50% of the total variation in PRC was significantly explained by a regression towards the mean effect of baseline estrogen ratio ($P = 0.001$). Figure 4 displays the linear relationship between PRC and baseline ratio for the HD group, and the lack of correlation in the case of the C + LD group.

From regression analysis, we found a significant adjusted difference in PRC between the two groups as long as baseline estrogen ratio was less than 2.92. Table I tabulates the differences and the corresponding 95% CIs for some selected values of baseline ratio.

DISCUSSION

The goal of this placebo-controlled, double-blind study was to determine a minimum effec-

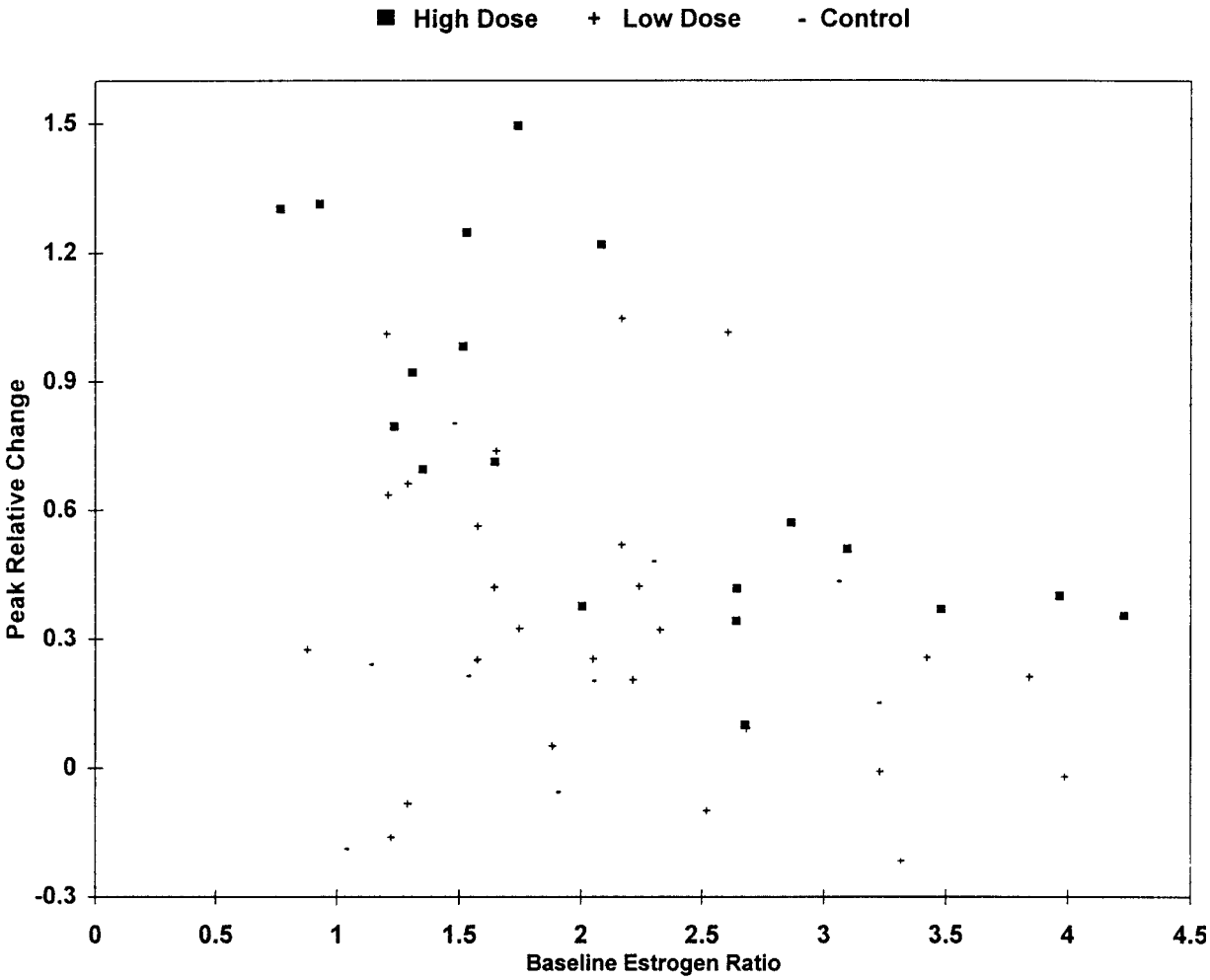

Fig. 4. Plot of peak relative change of urinary estrogen ratio vs. baseline value. Linear regression was significant only in the high-dose group: y = 1.38–0.29x, $P = 0.001$, $R^2 = 0.50$. For control + low-dose group, mean peak relative change ±SD = 0.32 ± 0.33.

tive and safe dose schedule of I3C that will result in a significant increase in the urinary estrogen metabolite ratio of 2-hydroxyestrone to 16α-hydroxyestrone. We have shown in a sample of 57 women that an appropriate choice of MED was 300 mg and that daily intake of I3C at this dose presented no significant toxicity in a 4-week trial. At this MED dose schedule, peak relative change of the estrogen metabolite ratio was significantly greater than that at the lower doses, and the difference was more pronounced for women with lower baseline ratios. However, there was no significant perturbation of the biomarker for women with high and presumably already protective baseline ratios. Menopausal status was not a significant factor for perturbation of the biomarker in our analysis, although there was a trend towards greater up-regulation of the ratio in the case of pre-menopausal women. A larger study should be conducted to confirm the findings reported here, particularly the lack of effect of menopausal status on the perturbation of the

TABLE I. Adjusted Differences in PRC of Urinary Estrogen Ratio Between High-Dose Group and Combined Control and Low-Dose Group*

Baseline ratio	Adjusted difference	95% CI Lower	95% CI Upper	*P* value
Q1 = 1.41	0.65	0.44	0.88	<0.001
M = 2.01	0.48	0.3	0.67	<0.001
Q3 = 2.66	0.29	0.1	0.49	0.004
C = 2.92	0.22	0	0.43	0.05

*Q1, M, and Q3 represent the first quartile, median and third quartile of baseline ratio, respectively. C represents the critical baseline ratio beyond which there was so significant difference in PRC between the two groups.

biomarker, and to identify an optimal effective dose schedule for a long-term breast cancer prevention trial.

ACKNOWLEDGMENTS

This study was supported in part by the Murray and Isabella Rayburn Foundation and by the Tiger Foundation.

REFERENCES

1. Wattenberg LW, Loub WD (1978): Inhibition of polycyclic aromatic hydrocarbon induced neoplasia by naturally occurring indoles. Cancer Res 38:1410–1413.
2. Telang NT, Suto A, Wong GYC, Osborne MP, Bradlow HL (1992): Induction by estrogen metabolite 16α-hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. JNCI 84:634–636.
3. Suto A, Bradlow HL, Wong GYC, Osborne MP, Telang NT (1993): Experimental down-regulation of intermediate biomarkers of carcinogenesis in mouse mammary epithelial cells. Breast Cancer Res Treat 27:193–202.
4. Bradlow HL, Sepkovic DW, Klug T, Osborne MP (1998): Application of an improved Elisa assay to the analysis of urinary estrogen metabolites. Steroids (in press).
5. Bradlow HL, Michnovicz JJ, Telang NT, Osborne MP (1991): Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis 12:1571–1574.
6. Grubbs C, Steele VE, Casebolt T, Juliane MM, Eto I, Whitaker LM, Dragnec KH, Kelloff GJ, Lubet RL (1995): Chemoprevention of chemically induced mammary carcinogenesis by indole-3-carbinol. Anti-Cancer Res 15: 709–716.
7. Osborne MP, Karmali RA, Hershcopf RJ, Bradlow HL, Kourides IA, Williams WR, Rosen PP, Fishman J (1988): Omega-3 fatty acids: Modulation of estrogen metabolism and potential for breast cancer prevention. Cancer Invest 6:629–631.
8. Bradlow HL, Hershcopf RJ, Martucci CP, Fishman J (1985): Estradiol 16α-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: A possible model for the hormonal etiology of breast cancer in humans. Proc Natl Acad Sci USA 82:6295–6299.
9. Taioli E, Bradlow HL, Garbers SV, Sepkovic DW, Osborne MP, Trachman J, Ganguly S, Garte SJ (1998): Cyp1A1 genotype, estradiol metabolism and breast cancer in African-Americans. Cancer Prev Detect (in press).
10. Niwa T, Swaneck G, Bradlow HL (1994): Alterations in estradiol metabolism in MCF-7 cells induced by treatment with indole-3-carbinol and related compounds. Steroids 58:523–527.
11. Tiwari RK, Li Guo, Bradlow HL, Telang NT, Osborne MP (1994): Selective responsiveness of human breast cancer cells to indole-3-carbinol, a chemopreventive agent. JNCI 86:126–131.
12. Michnovicz JJ, Bradlow HL (1990): Induction of estradiol metabolism by dietary indole-3-carbinol in humans. JNCI 82:947–949.
13. Bradlow HL, Michnovicz JJ, Halper M, Miller DG, Wong GYC, Osborne MP (1994): Long-term responses of women to indole-3-carbinol or a high fiber diet. Cancer Epidemiol Biomarkers Prev 3:591–595.