Long-Term Responses of Women to Indole-3-carbinol or a High Fiber Diet[1]

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Abstract

We test the hypothesis that the estrogen metabolite ratio 2-OH-estrone:estriol can be raised via dietary indole-3-carbinol (I3C) and that this higher ratio can be sustained over a 3-month test period. We also explore the possible role of pure fiber on estradiol metabolism. Using a randomized clinical trial with three arms, each containing 20 subjects, arm 1 received 400 mg/day of I3C daily for 3 months, arm 2 received 20 g of α-cellulose daily for the same time period as a source of added fiber, and arm 3 received a placebo dose. Blood levels of a variety of biochemical parameters were measured. The urinary 2-OH-estrone:estriol estrogen metabolite ratio was measured monthly at the same time of the menstrual cycle. While no changes were observed in the control and α-cellulose-treated arms, a substantial mean increase in the ratio was observed in the I3C-treated arm at month 1; that increase was maintained over the 3-month time period. Three of the 20 subjects in this I3C-treated group differed from the others in that no significant change in the metabolite ratio was observed at any time point. The results suggest that I3C can serve to increase the 2-OH-estrone:estriol metabolite ratio in a sustained manner without detectable side effects and that some individuals may be resistant to such change.

Introduction

Our ultimate goal is to test the hypothesis that an increased 2-OH-estrone:estriol ratio will act in a protective manner to decrease breast cancer incidence. Earlier studies have established that there are two mutually exclusive pathways of estrogen metabolism at C-2 and C-16α. Because the pool of estrogen in the body is limited (1), any increase in either of the two principal alternative pathways of estrogen metabolism will result in decreased formation of the opposite metabolite. Thus, markedly increasing 2-hydroxylation results in a decrease in the formation of 16α-hydroxylated metabolites and *vice versa*. Previous studies have demonstrated that 16α-hydroxylation is a risk factor for breast cancer (2, 3) and that the product of this reaction, 16α-hydroxyestrone, is genotoxic toward mammary cells based on a variety of parameters (4). Further evidence comes from studies in murine models which show a close correlation between the extent of 16α-hydroxylation and the incidence of mammary tumors (5). In light of these observations it had originally seemed desirable to directly decrease the extent of 16α-hydroxylation as a strategy for decreasing breast cancer risk. But attempts to directly decrease this reaction have been consistently unsuccessful because the enzyme effecting this reaction appears to be constitutive and not easily altered except by relatively draconian procedures.

Fortunately, however, the alternative reaction, 2-hydroxylation, has proved to be easily altered by a variety of methods (6–11). Parallel studies on 2-hydroxyestrone have shown that this compound is not genotoxic and does not promote proliferation (12) or cause cell transformation.[3] We have chosen for study I3C,[4] a constituent of cruciferous vegetables that specifically induces the enzyme P4501A1, which carries out 2-hydroxylation (13).

Our prior short-term studies have shown a substantial increase in the 2-OH-estrone:estriol metabolite ratio following administration of I3C for 6 days (11). Studies in mice with high endogenous rates of mammary tumors have shown that feeding indole-3-carbinol to these animals from an early age results in a marked decrease in the incidence of these tumors (14). Recent epidemiological studies by Kohlmeier and Dortchy (15), indicating that breast cancer rates are decreased in populations consuming large amounts of cabbage, a potent source of indole-3-carbinol, also support the results found in this study.

The studies described below were aimed at determining: (*a*) whether dietary intervention could maintain an increase in 2-hydroxylation during a longer human trial; (*b*) whether any potentially harmful side effects could be detected; and (*c*) whether the beneficial effects of so-called "high fiber" diets should be ascribed to the fiber *per se* or to the associated phytochemicals. In earlier acute studies (11) we were unable to determine either persistence or possible side effects of increased 2-hydroxylation. In addition, most prior studies have used vegetables as a source of mixed fiber and phytochemicals. A problem with such vegetable-based sources is that one cannot be certain whether the fiber or the associated phytochemicals found in these diets were responsible for the observed decrease in 16α-hydroxylation (14, 16). To circumvent this problem we have compared a

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[3] R. Tiwari, private communication; unpublished results from this laboratory.
[4] The abbreviation used is: I3C, indole-3-carbinol.

Table 1 Summary of baseline parameters on subjects from the three groups

	Indole-3-carbinol (n = 20)	Cellulose (n = 20)	Placebo (n = 20)
Age (yr)	37.8 ± 7.3[a] (25–51)	38.3 ± 5.6 (27–48)	36.1 ± 8.0 (18–53)
Height (m)	1.64 ± 0.06 (1.51–1.75)	1.62 ± 0.08 (1.42–1.77)	1.63 ± 0.07 (1.49–1.75)
Weight (kg)	57.9 ± 5.9 (48.2–72.7)	59.5 ± 5.6 (48.5–68.2)	57.3 ± 6.2 (45.5–67.3)
Body-mass index (m/kg^2)	21.6 ± 1.7 (19.3–24.5)	22.7 ± 2.5 (19.6–28.8)	21.6 ± 1.5 (19.9–24.7)

[a] Mean ± SD (range).

group of volunteers receiving 400 mg/day of I3C and a second group receiving 20 g/day of α-cellulose as a source of pure fiber for a 3-month trial period and a control population receiving a placebo dose.

Subjects and Methods

Subjects were recruited from the high-risk registry of the Strang Cancer Prevention Center. Sixty volunteers were randomly assigned to the three trial arms. Subjects were initially interviewed and screened for conditions or dietary habits that would interfere with the study; their general conditions are outlined in Table 1. Group 1 received capsules containing 400 mg of I3C/day, group 2 received 20 g packets of α-cellulose to be mixed with fruit juice and consumed daily, and group 3 received placebo capsules for the 3-month trial. Subjects who were accepted for this study were then started on the regime on the morning of the second to fourth day of the menstrual cycle of each individual. At this time they collected a urine sample in a bottle containing a 100-mg ascorbic acid tablet. When they brought in the urine samples they were given a bottle of capsules (placebo or I3C) or envelopes of cellulose depending on the group to which they had been assigned. For a period of 3 months they returned at the end of each menstrual cycle to deliver another morning urine sample and to receive their next monthly bottle of capsules or a supply of cellulose packets. At this same time they were also interviewed concerning any untoward symptoms or problems. At the start of the study and at the end of each month blood samples were also collected for the determination of the biochemical parameters listed in Appendix A. Biochemical measurements were carried out by the clinical chemistry laboratory at the Memorial Hospital in New York. Sex hormone-binding globulin measurements were carried out by Dr. W. Rosner at Roosevelt-St. Luke's Hospital. During the course of the study all of the volunteers were encouraged to maintain their normal diet.

Urinary estriol and 2-hydroxyestrone were measured by a radioimmunoassay procedure as described previously (17).

Results

The supplements were well tolerated by the subjects for the most part. A few subjects dropped out of group 2 because they found the cellulose suspension very unpleasant to consume. Examination of the initial values for the individual subjects showed a wide range of individual values for both metabolites. The mean values for the 2-OH-estrone:estriol ratio in the volunteers receiving I3C showed a substantial increase at the 1-month point, which was maintained during the second and third months (Table 2). In addition it was observed that 3 of the subjects showed a complete inability to respond to the dose of I3C used in this study with induction of estradiol 2-hydroxylation (Appendix B). The subjects on the fiber regime from the zero time samples to the final 3-month samples showed no significant perturbation, with a high degree of reproducibility from month to month, as did the control subjects in group 3. Using Hotelling T^2 statistics in the 1-sample analysis of repeated measures (18) we were able to demonstrate that the mean ratios in the 3 consecutive months on I3C treatment were not statistically different; therefore, the 3 monthly ratios can be regarded as replications of the posttreatment ratio for each participating woman. In fact, analysis of variance showed that 85% of the total variation in the posttreatment data could be explained by intersubject differences, so that the within-subject biological fluctuations were relatively small. The generalized one-sample paired t test (19) showed that the mean posttreatment ratio was significantly higher than the mean baseline ratio ($P = 0.0001$). Moreover, up-regulation of C-2-hydroxylation relative to C-16α-hydroxylation was independent of the baseline ratio. The clinical parameters showed no significant changes in any of the three subject groups other than a slight increase in cholesterol levels in the I3C group (Appendix A, Group 1).

Table 2 Changes in urinary estrogen index in three treatment arms

	2-OH-estrone:estriol			
	Baseline	Month 1	Month 2	Month 3
Indole-3-carbinol	0.72 ± 0.31	1.17 ± 0.61[a]	1.09 ± 0.52[a]	1.19 ± 0.57[a]
Cellulose	0.75 ± 0.57	0.72 ± 0.52	0.74 ± 0.38	not available
Placebo	0.73 ± 0.34	0.74 ± 0.32	0.67 ± 0.34	0.76 ± 0.43

[a] Hotelling T^2 statistics and generalized paired t test indicated that the results for all 3 months on indole-3-carbinol were the same and significantly different from the baseline. No difference was observed in the other two arms.

Discussion

Measurements of the 2-OH-estrone:estriol ratio showed that the response noted in our prior short-term studies was sustained over the 3-month period of this trial. Examination of the individual results showed that most of the subjects on I3C showed a significant and sustained increase in the metabolite ratio over the trial period. Three subjects (two with low initial metabolite ratios and one with a higher initial value) did not respond to I3C with an increase in the metabolite ratio. Several possible explanations exist for this failure to alter the ratio. These patients may require a larger dose of I3C to induce a response (*e.g.*, 600–1000 mg/day) or they may have a polymorphic form of the enzyme which is unable to induce 2-hydroxylation. An ongoing study in an Israeli Kibbutz, in which the subjects were fed cruciferous diets for 1, 3, or 5 days in a quantity calculated to contain 400 mg of I3C, also found a few subjects who failed to respond to the vegetable diet.[5] In a private communication, Dr. A. B. Okey (Univ. of Toronto) has reported a similar finding in studying P-450 activity in the placentae of women who smoked during pregnancy. Furthermore, al-

[5] Unpublished data.

though all of our patients insisted that they had consumed the capsules faithfully it is also possible that the subjects who did not respond failed to take their capsules consistently.

Regardless of the mechanism involved, the inability to increase 2-hydroxylation may indicate that there is a subset of individuals who are at greatest risk for breast cancer and who should be followed closely. Further studies on the effects of a longer trial period are planned. Experimental studies indicate that increased levels of 2-OH-estrone reduce the risk of mammary tumors by increasing the ratio of 2-OH-estrone/16α-estrone (20). The results showed that I3C has only minor effects on the clinical parameters chosen as measures of potential side effects of I3C intake. Careful questioning of the patients in the I3C arm revealed no significant differences other than a slight increase in gastrointestinal motility and a decrease in complaints of constipation in a few subjects. These results support the feasibility of using diet-derived compounds to alter estrogen metabolism in a direction that should exert a protective effect against breast cancer.

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Appendix A

	Group 1: Indole-3-carbinol			
	Baseline	Month 1	Month 2	Month 3
Enzymes				
Hemoglobin	12.9 ± 0.7[a]	12.7 ± 0.8	12.5 ± 0.7	12.7 ± 0.6
Platelets	290.5 ± 56.4	287.1 ± 40.6	293.4 ± 63.5	287.8 ± 55.0
BUN	14.0 ± 3.3	12.9 ± 2.4	13.3 ± 2.1	12.9 ± 2.0
Bilirubin	0.6 ± 0.5	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.2
Uric acid	4.8 ± 0.8	4.2 ± 0.7	4.3 ± 0.9	4.3 ± 1.1
Total protein	6.6 ± 0.3	6.6 ± 0.4	6.5 ± 0.4	6.6 ± 0.3
Albumin	4.5 ± 0.2	4.5 ± 0.2	4.4 ± 0.2	4.4 ± 0.2
Calcium	9.2 ± 0.3	9.2 ± 0.4	9.1 ± 0.4	9.1 ± 0.4
Phosphate	3.7 ± 0.5	3.7 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
SGOT	18.4 ± 4.2	19.8 ± 4.2	20.3 ± 5.2	20.8 ± 6.1
LDH	156.2 ± 14.7	155.8 ± 20.5	153.1 ± 19.9	151.9 ± 24.5
ALK PHOS	49.8 ± 13.6	47.6 ± 12.9	44.6 ± 12.1	46.8 ± 13.2
Cholesterol profile				
Total cholesterol	186.9 ± 39.4	191.1 ± 36.2	188.3 ± 37.2	198.1 ± 40.4
HDL	59.7 ± 12.4	57.3 ± 11.8	55.7 ± 10.9	58.3 ± 13.4
LDL	112.1 ± 37.21	18.3 ± 36.1	116.0 ± 35.5	123.4 ± 39.1
TC/HDL	3.2 ± 0.9	3.5 ± 1.0	3.5 ± 1.1	3.6 ± 1.3
Endocrinological parameters				
TSH	2.0 ± 2.3	1.5 ± 1.8	1.5 ± 1.5	1.6 ± 1.8
Estradiol	109.5 ± 74.6	112.9 ± 57.3	95.2 ± 81.3	80.9 ± 40.8
SHBG	65.4 ± 34.0	71.5 ± 36.9	68.9 ± 34.5	70.4 ± 37.4
Urinary estrogens				
2-OH-estrone	9.6 ± 6.1	12.5 ± 8.1	10.4 ± 5.0	13.3 ± 9.4
Estriol	13.9 ± 7.1	11.3 ± 5.3	10.3 ± 3.9	12.2 ± 7.7
Estrone	13.6 ± 10.1	13.7 ± 10.0	12.1 ± 9.5	11.9 ± 7.4
Sum Es	37.0 ± 20.8	37.7 ± 17.5	32.8 ± 12.2	37.4 ± 20.6
2-OH-estrone:estriol	0.72 ± 0.31	1.17 ± 0.61	1.09 ± 0.52	1.19 ± 0.57
Menses (Days)	27.2 ± 3.2	27.1 ± 2.6	27.3 ± 2.6	27.5 ± 2.4
	Group 2: Cellulose			
Enzymes				
Hemoglobin	12.8 ± 0.7	12.7 ± 0.8	12.4 ± 0.8	11.4 ± 0.8
Platelets	264.1 ± 72.1	265.0 ± 65.6	279.6 ± 84.2	277.4 ± 89.5
BUN	12.4 ± 2.7	11.9 ± 2.5	12.6 ± 2.9	13.2 ± 3.4
Bilirubin	0.6 ± 0.4	0.6 ± 0.4	0.6 ± 0.4	0.5 ± 0.3
Uric acid	4.1 ± 0.7	3.9 ± 0.8	4.0 ± 0.8	4.0 ± 1.0
Total protein	6.8 ± 0.4	6.7 ± 0.4	6.1 ± 0.4	6.6 ± 0.4
Albumin	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.3
Calcium	9.3 ± 5.4	22.0 ± 3.9	22.0 ± 3.7	20.9 ± 4.3
LDH	168.3 ± 34.0	164.3 ± 28.1	157.8 ± 29.6	159.6 ± 28.3
ALK PHOS	53.2 ± 11.8	51.6 ± 11.7	52.9 ± 12.0	50.9 ± 8.9
Cholesterol profile				
Total cholesterol	189.1 ± 33.0	186.8 ± 31.8	187.7 ± 33.2	190.6 ± 35.8
HDL	59.9 ± 10.6	59.8 ± 9.6	59.0 ± 9.7	61.6 ± 11.7
LDL	109.5 ± 32.1	106.7 ± 30.2	110.5 ± 34.4	113.3 ± 36.1
TC/HDL	3.3 ± 0.8	3.2 ± 0.7	3.3 ± 0.8	3.2 ± 1.0
Endocrinological parameters				
TSH	1.8 ± 1.2	1.5 ± 1.5	1.9 ± 2.1	1.4 ± 1.0
Estradiol	70.1 ± 36.9	75.2 ± 44.9	79.2 ± 43.0	95.9 ± 53.7
SHBG	65.4 ± 17.8	64.4 ± 17.7	59.7 ± 18.0	58.9 ± 15.9
Urinary estrogens				
2-OH-estrone	8.6 ± 5.5	7.8 ± 4.9	8.6 ± 4.4	9.4 ± 5.6
Estriol	13.1 ± 6.9	12.5 ± 7.4	13.2 ± 7.9	13.8 ± 7.5
Estrone	9.4 ± 3.9	9.7 ± 5.5	9.8 ± 4.5	11.9 ± 6.5
Sum Es	31.3 ± 10.1	30.0 ± 13.0	31.6 ± 11.6	35.1 ± 15.4
2-OH-estrone:estriol	0.87 ± 0.71	0.80 ± 0.64	0.86 ± 0.67	0.79 ± 0.42
Menses (Days)	29.1 ± 6.1	28.1 ± 3.5	27.6 ± 2.9	29.5 ± 6.6

Appendix A (Continued)

	Group 3: Placebo			
Enzymes				
Hemoglobin	12.6 ± 1.2	12.7 ± 0.6	12.9 ± 0.6	12.4 ± 0.9
Platelets	259.0 ± 74.4	276.7 ± 66.1	277.5 ± 71.1	260.8 ± 72.5
BUN	12.6 ± 2.3	12.4 ± 2.7	12.0 ± 2.8	12.0 ± 3.5
Bilirubin	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Uric acid	4.0 ± 1.1	4.2 ± 1.2	4.1 ± 0.9	4.2 ± 0.9
Total protein	6.7 ± 0.4	6.7 ± 0.3	6.8 ± 0.4	6.7 ± 0.5
Albumin	4.5 ± 0.3	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.3
Calcium	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3
Phosphate	4.1 ± 0.6	3.9 ± 0.5	4.0 ± 0.5	4.1 ± 0.4
SGOT	21.2 ± 5.0	21.4 ± 8.6	24.5 ± 16.8	21.4 ± 6.6
LDH	208.9 ± 219.9	147.5 ± 13.3	156.5 ± 24.6	154.1 ± 18.8
ALK PHOS	50.3 ± 13.7	47.7 ± 13.8	47.9 ± 13.0	47.9 ± 13.3
Cholesterol profile				
Total cholesterol	183.8 ± 35.9	184.5 ± 33.3	133.8 ± 28.8	178.2 ± 31.9
HDL	56.5 ± 11.4	59.2 ± 12.9	56.9 ± 13.0	56.4 ± 12.3
LDL	110.9 ± 29.0	110.5 ± 32.0	111.7 ± 22.9	106.4 ± 24.0
TC/HDL	3.3 ± 0.7	3.2 ± 0.8	3.3 ± 0.7	3.2 ± 0.6
Endocrinological parameters				
TSH	1.6 ± 0.7	1.6 ± 1.3	1.7 ± 1.0	1.7 ± 1.0
Estradiol	77.1 ± 35.7	82.9 ± 43.8	64.8 ± 134.8	81.8 ± 50.3
SHBG	68.2 ± 26.2	62.9 ± 19.2	69.2 ± 20.2	74.4 ± 22.5
Urinary estrogens				
2-OH-estrone	9.2 ± 5.4	9.1 ± 4.1	8.4 ± 3.4	10.3 ± 5.3
Estriol	14.3 ± 11.4	13.0 ± 6.3	15.0 ± 9.1	16.2 ± 9.5
Estrone	11.6 ± 7.6	11.6 ± 5.0	10.6 ± 4.6	11.4 ± 5.0
Sum Es	35.6 ± 19.3	33.8 ± 10.2	34.1 ± 12.2	37.8 ± 14.5
2-OH-estrone:estriol	0.75 ± 0.36	0.78 ± 0.32	0.68 ± 0.34	0.77 ± 0.40
Menses (Days)	29.3 ± 3.3	27.7 ± 4.5	23.9 ± 4.4	29.9 ± 5.3

[a] Mean ± SD.
[b] BUN, blood urea nitrogen; SGOT, serum glutamic-oxaloacetic transaminase; LDH, lactate dehydrogenase; ALK PHOS, alkaline phosphatase; HDL, high density lipoprotein; LDL, low density lipoprotein; TC/HDL, total cholesterol:high density lipoprotein ratio; TSH, thyroid-stimulating hormone; SHBG, sex hormone-binding globulin.

Appendix B Effect of I3C on urinary estrogen metabolite ratios measured over three consecutive months

Subjects	2-OH-estrone:estriol ratio			
	Baseline	Month 1	Month 2	Month 3
1	0.878	1.674	1.209	1.376
2	0.845	1.463	1.289	1.202
3	1.028	1.875	1.515	1.642
4	1.380	2.015	2.030	2.099
5[a]	0.341	0.370	0.504	0.324
6	0.577	1.836	1.633	1.858
7	0.331	0.604	0.423	0.699
8	0.785	1.149	1.025	1.885
9	0.589	0.626	0.670	0.860
10	0.791	1.306	0.877	1.142
11	0.266	0.571	0.694	0.544
12	0.675	0.938	0.763	0.983
13	0.526	0.580	1.500	1.608
14	0.580	1.413	1.447	1.136
15	0.714	0.693	1.012	0.927
16[b]	1.348	1.372	1.300	1.167
17	0.553	0.973	0.778	0.510
18	1.100	2.652	2.182	2.391
19[a]	0.622	0.721	0.607	0.629
20	0.445	0.619	0.355	0.722

[a] Low level nonresponders to I3C.
[b] High level nonresponder to I3C.

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