

## EFFICACY OF DRIED CRUCIFEROUS POWDER FOR RAISING THE 2/16 HYDROXYESTROGEN RATIO

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### ABSTRACT

Urinary 2-hydroxyestrogens, 16- $\alpha$ -hydroxyestrone, and the 2/16 hydroxyestrogen ratio were determined in 13 premenopausal women before and after the addition of dietary supplements containing 3.6 g of powdered organic Brussels sprouts and organic kale for 90 days. Twelve of the subjects had an initial 2/16 hydroxyestrogen ratio that was below the cutoff of 2.0. Lower 2/16 hydroxyestrogen ratios have been associated with increased risk for cancers of estrogen-sensitive tissues. 11 of the 13 subjects showed positive increases of their ratios, and the three subjects with the lowest initial ratios had an average increase of 500%. The results demonstrate that dehydrated whole brassicaceae supplementation can significantly raise the 2/16 hydroxyestrogen ratio in premenopausal women.

### INTRODUCTION

Incidence of breast cancer increases with total estrogen exposure, and urinary concentrations of estrogen metabolites are related to risk of breast cancer in women.<sup>1,2</sup> The cancer effects derive largely from the increased estrogen load and direct DNA damaging potential of the 16- $\alpha$ -hydroxyestrone.<sup>3-6</sup> This compound and the 2-hydroxylated metabolites of estrone and estradiol constitute the principal urinary products of estrogen. The risk of cancers in estrogen-sensitive tissues increases in proportion to the percentage of estrogen converted to 16- $\alpha$ -hydroxyestrone, usually expressed as the ratio of urinary 2-hydroxyestrone plus 2-hydroxyestradiol to 16- $\alpha$ -hydroxyestrone (the 2/16 ratio). Cancer risk increases in proportion to the 2/16 ratio depression below 2.0.<sup>7</sup>

Research in this area was originally fueled by the epidemiological observation that risk of breast cancer was lowered by consumption of whole cruciferous (Brassica) vegetables.<sup>8-10</sup> Phytochemicals from crucifers, including indole-3-carbinol (I3C) (and the I3C derivative, diindolylmethane (DIM)) were found to stimulate the synthesis of the 1A1 class of cytochrome P450 enzymes (CYP1A1), resulting in increased rates of 2-hydroxylation.<sup>11-14</sup> Increased hepatic 2-hydroxylation

decreases the flux of estrogens through the alternative 16- $\alpha$ -hydroxylation pathway in liver and extrahepatic tissues, raising the 2/16 ratio.

A number of recent studies and meta-analyses have shown that the beneficial physiological effects of whole fruit and vegetables, including the crucifers, cannot be attributed to only one component in the food.<sup>15-21</sup> Such effects include detoxification and cancer protection. Thus, factors other than CYP1A1 levels may affect the 2/16 ratio. For example, a significant portion of the hydroxylated derivatives undergo methylation catalyzed by catechol-O-methyltransferase, becoming methoxy compounds that are excreted.<sup>22</sup> Altering folate, or methyl donor compound (methionine, glycine) intake can change methylation rates. Dietary fiber can decrease the rate of enterohepatic circulation, lowering total estrogen load.<sup>23</sup> However, since the CYP1A1 stimulation is attributed to the presence of indolyl-3-carbinol (I3C) or its active conjugate diindolylmethane (DIM), emphasis has been centered on nutritional supplements that contain only these compounds.<sup>24</sup> Such single compound extracts may lose effectiveness through the lack of potential benefits from the entire range of natural compounds found in whole crucifers. In addition, relatively large doses of I3C may fail to undergo complete conversion to the active metabolite.



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DIM, especially in individuals with low stomach acid output.<sup>25</sup> While the protective, anti-cancer effect of high fruit and vegetable intake has been demonstrated consistently, single nutrient supplementation has generated conflicting results.<sup>26</sup>

Although adding cruciferous vegetables to the diet can improve abnormally low 2/16 ratio status, some patients cannot make the necessary dietary adjustments. Since food preference or food availability may limit compliance with dietary instructions, a dietary supplement may offer an effective alternative. The efficacy of using dietary supplements of desiccated whole cruciferous vegetables to improve low 2/16 ratios has not been studied. The purpose of this study was to determine whether the use of a supplement consisting of dried organic Brussels sprouts and kale could elicit a positive effect to raise the 2/16 ratio in premenopausal women.

## METHODS

### PARTICIPANTS

This study was an open trial, as no matched placebo group was used. Forty-five women were selected for

screening as possible candidates to participate in the study after completing an approved informed consent form. They agreed to comply with the study outline which included maintaining constant status of Brassica vegetable and alcoholic beverage intake and constant use of any nutritional or herbal products. They also agreed to maintaining levels of exercise and to using only medications that were medically prescribed. Of the 23 women who began the study, 13 premenopausal women completed the study. Ages ranged from 34 to 47 years and stated compliance were 80% or higher regarding regular consumption of supplements and other requirements.

### TESTING

First morning urine specimens were collected and promptly frozen prior to overnight shipment to the testing laboratory. Measurements of 2-hydroxyestrone + 2-hydroxyestradiol and 16- $\alpha$ -hydroxyestrone were performed by Metamatrix Clinical Laboratory, Duluth, GA using an FDA-approved kit based on enzyme immunoassay methodology. Hydroxyestrogen concentrations were expressed as ng/mg creatinine, and 2/16 ratios were calculated as the sum of the two 2-hydroxy derivative

Subject	Creatinine		2-Hydroxyestrogens		16-Hydroxyestrone		Total Measured Hydroxyestrogens		2/16 Ratio		% Change
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
23	33	166	5.2	6.7	16.1	2.2	21.3	8.9	<b>0.32</b>	<b>3.05</b>	<b>843%</b>
12	63	62	7.9	10.5	14.9	7.3	22.8	17.8	<b>0.53</b>	<b>1.44</b>	<b>171%</b>
25	246	86	3.2	8.9	5.7	2.7	8.9	11.6	<b>0.56</b>	<b>3.30</b>	<b>487%</b>
3	155	208	10.2	26.3	16.9	33.1	27.1	59.4	0.60	0.79	32%
2	108	109	9.9	12.6	15.6	16.5	25.5	29.1	0.63	0.76	20%
15	153	119	11.8	15.2	10.8	9.8	22.6	25.0	1.09	1.55	42%
6	187	94	12.6	21.1	9.0	20.4	21.6	41.5	1.40	1.03	-26%
19	69	79	9.0	23.4	6.4	10.1	15.4	33.5	1.41	2.32	65%
27	134	115	5.7	21.2	3.4	4.5	9.1	25.7	1.68	4.71	181%
20	142	129	14.7	16.4	8.4	6.3	23.1	22.7	1.75	2.60	49%
21	81	88	35.4	23.4	19.1	8.0	54.5	31.4	1.85	2.93	58%
26	140	53	24.0	20.9	12.5	5.7	36.5	26.6	1.92	3.67	91%
14	148	174	26.8	14.9	10.8	9.9	37.6	24.8	2.48	1.51	-39%
Avg	126	109	12.5	17.2	11.6	10.6	24.0	27.8	1.1	2.3	168%

**Table 1.** Rows are sorted from low to high values of the initial 2/16 ratio. Results from first morning urine specimen analysis of the 2-hydroxyestrone + 2-hydroxyestradiol sum and 16- $\alpha$ -hydroxyestrone before and after the 90-day supplementation with cruciferous vegetable powder. Total measured hydroxyestrogen values were calculated as the sum of 2-hydroxyestrogens and 16-hydroxyestrone. Initial and final ratios and percentages of increase for the three lowest ratios are shown in bold font.



concentrations divided by the 16- $\alpha$ -hydroxyestrone concentration. 12 of the 13 subjects had 2/16 hydroxyestrogen ratios less than 2.0, the value below which cancer risk increases. Three of them had ratios less than 0.6, where even greater risk has been found.

**INTERVENTIONS**

Subjects took a dietary supplement that consisted of desiccated, powdered and encapsulated organically-grown Brussels sprouts and kale. Each capsule contained 300 mg of each powder. Subjects were instructed to take the 3 capsules with meals twice daily, providing for a daily intake of 3.6 g of desiccated whole vegetables. This amount of dehydrated product corresponds to approximately 36 g of fresh vegetables. The subjects used the supplements for 90 days and submitted first morning urine specimens again after completion of the last supplement dose.

**RESULTS**

No side effects were reported by any subject. Shifts in estrogen metabolism were demonstrated by higher 2/16 ratios found for 11 of the 13 subjects (Table 1). The subject with the lowest ratio of 0.32 had an almost 10-fold increase to 3.05, well above the cancer risk category cutoff of 2.0. The three subjects with the lowest initial ratios showed an average improvement of 500%. The overall average ratio

improvement was 168%. Ratio values increased in eleven subjects due to increased 2-hydroxyestrogens, and in 9 subjects due to lowered 16-hydroxyestrone excretion. Six individuals shifted from ratio values below 2.0 to values well above the 2.0 cutoff, including two of the three with initial ratios less than 0.6. Paired sample t-test analysis of initial and final 2/16 ratios for the 13 subjects showed significant increase in mean values, with a P = 0.01 (Figure 2).

**DISCUSSION**

The risk of cancers in estrogen-sensitive tissues increases as urinary 2/16 hydroxyestrogen ratios fall below values of about 2.0. The cancer risk effect is due to increased estrogenic stimulation of the proliferative stage of carcinogenesis by 16-hydroxyestrone.<sup>7</sup> Previous studies have demonstrated that higher 2/16 ratios are associated with diets high in cruciferous vegetables<sup>8</sup> and consumption of concentrated extracts of cruciferous vegetables.<sup>27</sup> However, in routine clinical practice, patient compliance with the necessary dietary changes can be poor. The current study showed that twice daily consumption of 3 capsules containing a total of 600 mg of equal parts of dehydrated organic Brussels sprouts and kale for 90 days is effective for raising the 2/16 ratio in premenopausal women with initially low ratios. High variability in responses toward increased 2-hydroxylation of estrogens

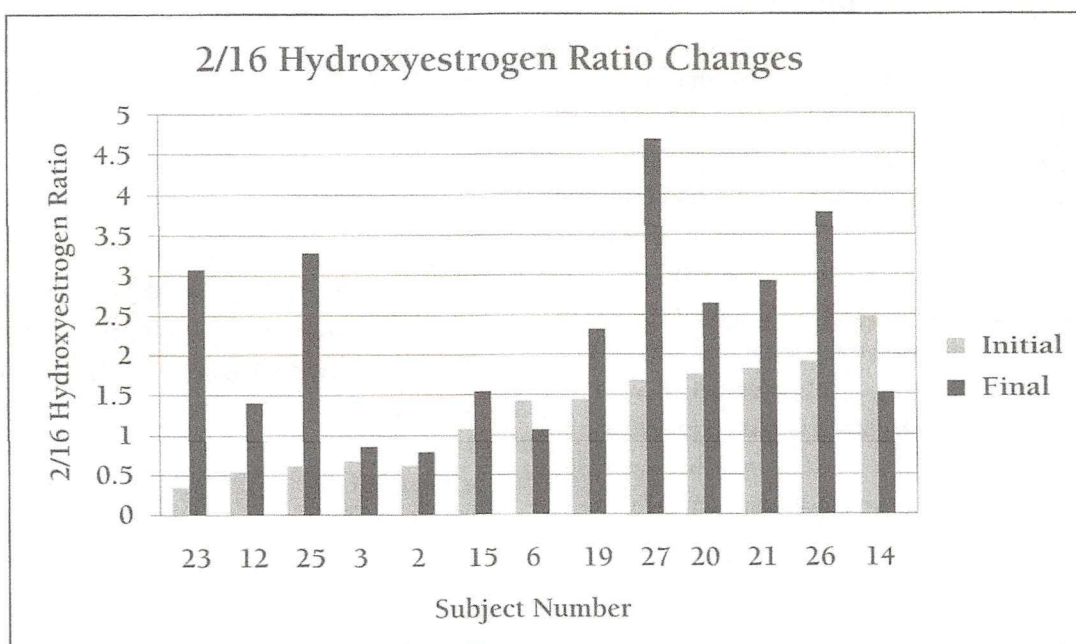
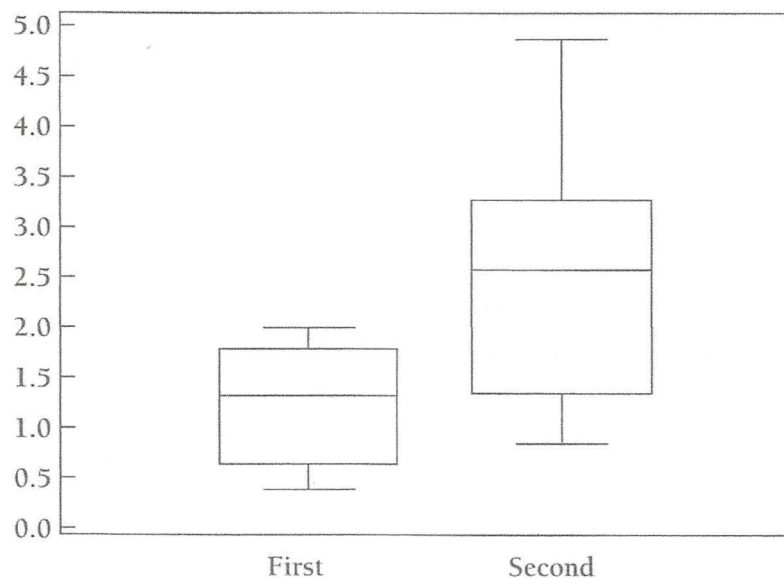


Figure 1. Shifts from initial to final 2/16 hydroxyestrogen ratios produced by cruciferous powder dietary supplements. Each line represents the initial and final 2/16 hydroxyestrogen values for one subject. The strong trend to move from lower to higher values is apparent, though 2 subjects showed final values less than their initial levels.





**Figure 2.** Means and error bars showing 95% confidence intervals from paired t-test analysis of the 13 initial and final 2/16 ratios. The two-tailed probability was 0.010, indicating statistical significance of the increase in mean values from 1.1 to 2.3. The large variability is consistent with known individually variable responses for cytochrome P450 induction.

is expected from previous studies that have demonstrated wide individual variation for induction of cytochrome P450 enzyme activities.<sup>28, 29</sup>

The finding that 11 of the 13 subjects in this study (who reported 80% compliance with the study protocol) showed strongly positive increases in their 2/16 ratios is unexpected. Even more unexpected was the 500% average rise of ratio values found for the three subjects with the lowest initial ratios. Such results that exceed those found for concentrated I3C extracts are suspected to be due to synergistic effects of multiple phytonutrients in the whole foods on hepatic cytochrome P450 stimulation. The magnitude of hydroxyestrogen changes were comparable with those found in women treated with up to 400 mg of I3C extract, though the desiccated whole food powders are estimated to contain less than 1/100th the amount of I3C.<sup>30</sup> Although the brassicaceae are uniquely rich in I3C, they also contain other classes of potentially beneficial compounds, including flavonoids and hydroxycinnamic acids.<sup>31</sup> Though neither the 2 nor 16 hydroxyestrogens showed a statistically significant positive change individually, the 2/16 hydroxyestrogen ratio improved.

These results demonstrate potential benefits from the entire range of natural compounds found in whole crucifers compared to single compound extracts, such as DIM and I3C. An additional benefit is the lower concern

about lack of conversion to active forms and lower risk of side effects. For women unable to make the dietary changes needed to increase their 2/16 ratio, the option of using a dried vegetable supplement can be effective. Also of interest is that, although the lowest initial ratio values showed very large percentage changes, the effects diminish as the initial ratio increases. Thus, there appears to be no reason for concern about potential over-stimulation of 2-hydroxylation at the dosages used for 90 day intervals. Extended suppression of 16- $\alpha$ -hydroxylation has the potential to influence osteoporosis in postmenopausal women.<sup>32</sup>

## CONCLUSIONS

Use of dietary supplements containing 3.6 g of dehydrated organic Brussels sprouts and kale is effective for increasing the 2/16 hydroxyestrogen ratio in premenopausal women. Individuals with the lowest ratios have the most dramatic increases. Further study is needed with larger and more diverse populations to verify the effects seen in this study and to determine the effectiveness of such interventions in populations with various ethnic and age differences. Also, more detailed studies using specified combinations of phytonutrients may reveal the origin of the apparent synergistic effects produced by the combination of Brussels sprouts and kale.

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