

Estrogen Metabolism and the Diet-Cancer Connection: Rationale for Assessing the Ratio of Urinary Hydroxylated Estrogen Metabolites

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Abstract

Estrogens are known for their proliferative effects on estrogen-sensitive tissues resulting in tumorigenesis. Results of experiments in multiple laboratories over the last 20 years have shown that a large part of the cancer-inducing effect of estrogen involves the formation of agonistic metabolites of estrogen, especially 16 α -hydroxyestrone. Other metabolites, such as 2-hydroxyestrone and 2-hydroxyestradiol, offer protection against the estrogen-agonist effects of 16 α -hydroxyestrone. An ELISA method for measuring 2- and 16 α -hydroxylated estrogen (OHE) metabolites in urine is available and the ratio of urinary 2-OHE/16 α -OHE (2/16 α ratio) is a useful biomarker for estrogen-related cancer risk. The CYP1A1 enzyme that catalyzes 2-hydroxyestrone (2-OHE1) formation is inducible by dietary modification and supplementation with the active components of cruciferous vegetables, indole-3-carbinol (I-3-C), or diindolylmethane (DIM). Other dietary components, especially omega-3 polyunsaturated fatty acids and lignans in foods like flaxseed, also exert favorable effects on estrogen metabolism. Thus, there appear to be effective dietary means for reducing cancer risk by improving estrogen metabolism. This review presents the accumulated evidence to help clinicians evaluate the merit of using tests that measure estrogen metabolites and using interventions to modify estrogen metabolism.

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Introduction

Clinical management of women's health issues is continually changing. First there was hormone replacement therapy with estradiol from pregnant mares' urine. Then there was balancing estradiol with progestins and, in some users, with estriol. Next the soy influence led the phytoestrogen wave. Now it has been found that other estrogen metabolites influence cancer and ways to manage these metabolic issues are becoming evident.

This review focuses on the clinical impact of knowledge regarding the metabolic fates of estrogens. With this knowledge, one can move from the notion that postmenopausal women should try to maintain high plasma estrogen levels to treatment that can maximize the positive tissue maintenance effects while minimizing the risk of cancer. The impact of the most potent carcinogenic metabolites may be controlled in most patients with cruciferous vegetables and their active

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Table 1. *Estrogen-Cancer Associations*

Year	Finding	Ref.
1896	Ovary removal gives remission of breast cancer.	1
1979	Estrogen use linked to endometrial cancer.	8
1980	Effect of estriol on mammary carcinomas debated.	9,10
1985	Estrogen is cause of endometrial cancer and likely is a factor in breast cancer.	11
1995	Estrogens promote mammary cancer in rodents and exert cell proliferative effects in humans.	12
1995	Family history, sex hormones, diet, lifestyle, and environmental exposure are factors associated with breast cancer incidence.	2
1996	Differences in exposure to xenoestrogens like insecticides, weed killers, synthetic estrogens, and aromatic hydrocarbons may explain the five-fold higher rate of cancer in Canadian women compared to women in Asia.	6
1997	Multiple prospective studies strongly suggest that breast cancer risk in postmenopausal women is associated with relatively high concentrations of endogenous estradiol.	13
2000	Women with family history of breast cancer who used early oral contraceptive formulations have particularly high risk for the disease.	14
2001	Hormone replacement therapy is conclusively linked with breast cancer.	15

components. Improving omega-3 fatty acid intake can provide additional metabolic protection for estrogen metabolism. With the knowledge of the effects of diet on estrogen metabolites, women may reduce their risk of estrogen-associated cancer.

Cancer Associations

The causes of cancer are complex, even when the issue is isolated to cancers in estrogen-sensitive tissues. The causes may be categorized as either factors that result in genotoxic DNA damage or factors that stimulate cell proliferation, development, and growth. Estrogen production is

clearly associated with cancer in estrogen-sensitive tissues. An early experiment that revealed the association of estrogen production and cancer was the remission of breast cancer in premenopausal women by surgical removal of the ovaries.¹ The close relationship between the risk of breast cancer and exposure to estrogen has been reviewed.^{2,3}

In the uterus, estrogen triggers the proliferation of endometrial cells during each month of the menstrual cycle, followed by death of these cells during menstruation. Similarly, during each menstrual cycle, estrogen normally triggers the proliferation of cells that form the inner lining of the ducts in the breast. Over a span of 40 years, from puberty to menopause, hundreds of cycles of cell division and cell death will occur.

These repeated cycles of estrogen-induced cell division tend to increase the risk of developing cancer in two ways: estrogen can stimulate the division of uterine or breast cells that already have DNA mutations (oncogene or virus^{4,5} initiated), and it also increases the chances of developing new, spontaneous mutations (from carcinogens⁶ or radiation). Whether the mutations are inherited or spontaneous, estrogen-driven proliferation increases the number of altered cells that can ultimately lead to the development of uterine or breast cancer.⁷ A historical perspective on the development of the estrogen-cancer association from early observations to recent conclusive reports is presented in Table 1.

Limiting exposure to environmental estrogens can reduce cancer risk. For example, estrogen activity has been found for nonylphenol and bisphenol. Nonylphenol is an antioxidant used in the manufacture of plastic, detergents, toiletries, lubricants, and spermicides. Bisphenol is leached from polycarbonate plastics when they are heated.¹⁶ Other xenoestrogens include DDT, methoxychlor, aromatic hydrocarbons, and the common weed killer Atrazine that is widely used on corn crops.

It is too early to calculate cancer risk factor associations with specific or total xenoestrogen exposures, but the laboratory evidence of estrogen-like effects of many xenobiotics is clear. The evidence points to the xenobiotic contribution to excessive cell proliferation due to the total load

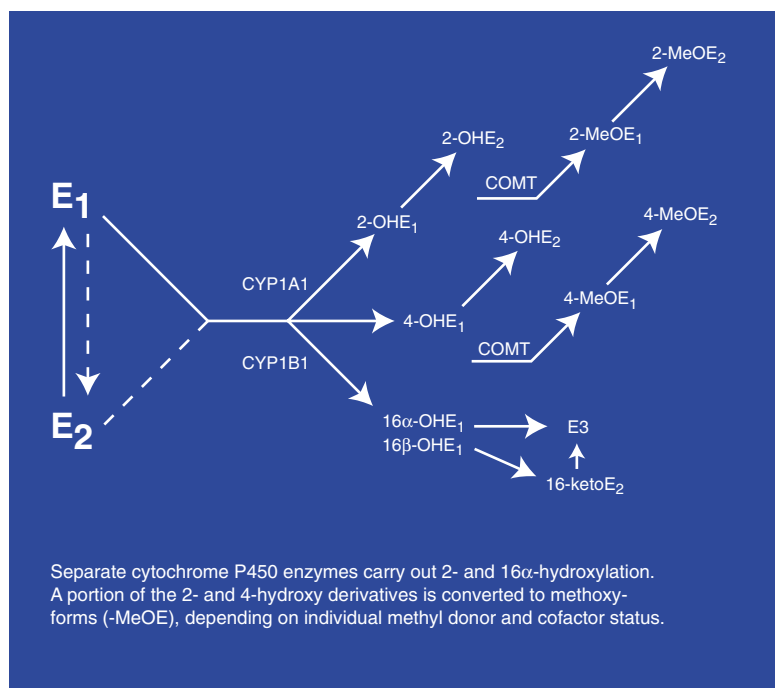
of estrogen-receptor stimulators. In addition, some compounds like DDT can exert direct effects on procarcinogenic estrogen metabolites discussed below.¹⁷ Adjustment of lifestyle to reduce xenoestrogen exposure is one step a woman can take to control total estrogen exposure.

Metabolic Fate of Estrogens

Normal premenopausal women produce several hundred micrograms of estradiol (E2) daily. A portion of the roughly 10^{17} newly synthesized molecules find their way to binding sites in the nucleus and other organelles of many tissues. Once bound to estrogen receptors, the estrogen hormones elicit an increased rate of DNA synthesis, resulting in gene transcription and cell division. Meanwhile, a similar number of estrone/estradiol molecules are removed from the body pool, maintaining a relatively constant stimulation of cell division in estrogen-sensitive tissues. Much of the estradiol is converted into estrone (E1) and estriol (E3), but these are only two of the best-known metabolites.

The half-life of E2 is about three hours.¹⁸ Its removal is accomplished by irreversible conversion into metabolites that may be passed into urine or bile. There are multiple pathways that convert E2 to products that have widely different biological activities. Some products are powerful carcinogens while others act as estrogen antagonists. The relative amounts of these metabolites control the overall cancer risk from estrogen exposure.

Oxidation to form hydroxy derivatives is a principal route of endogenous steroid metabolism (Figure 1). Isoenzymes of the cytochrome p450 (CYP) class can insert hydroxyl groups at the 2-, 4-, or 16- positions of E1 (Figure 2). The iso-enzyme that catalyzes 2-hydroxylation of E2 (CYP1A1) is an inducible enzyme. It is formed in greater amounts in hepatic microsomes in response to dietary ingredients and cigarette smoke. A separate enzyme, CYP1B1, catalyzes 16 α -hydroxylation. This enzyme is not inducible by diet, but xenobiotic carcinogens and pesticides may stimulate its activity.⁷ As a result, a preferential increase in 2-hydroxylation can occur through dietary manipulation.

Figure 1. Catabolism of Estradiol

After estrone hydroxylation, the various poly-hydroxy derivatives are conjugated with glucuronate or sulfate, or methylation occurs prior to excretion in urine. The catechol-O-methyl transferase (COMT) enzymes that catalyze the methylation reactions require S-adenosyl methionine. A portion of conjugated and unconjugated steroids also passes into bile, some of which may be reabsorbed via enterohepatic circulation. Lower intestinal reuptake rates can explain why total estrogen loads are decreased by high fiber diets and especially by the lignans contained in flax seed.

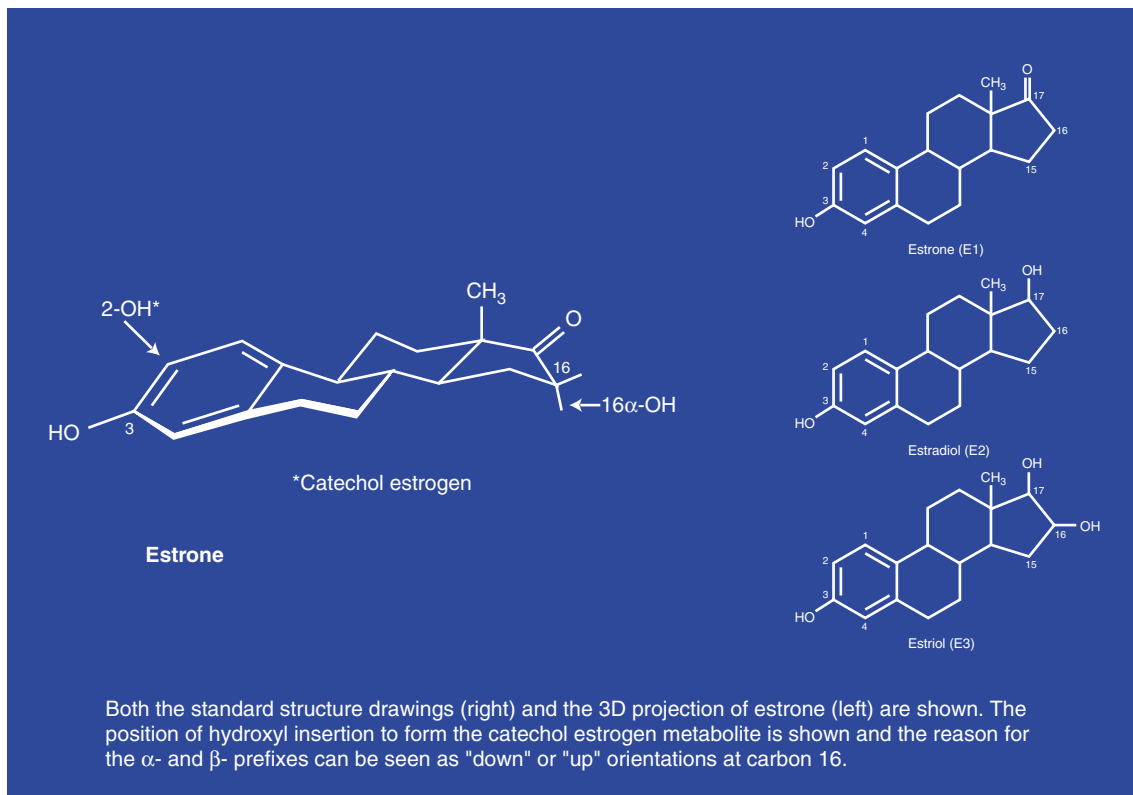
The principal hydroxylation products are 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol (4-OHE2), and 16 α -hydroxyestrone (16 α -OHE1). The 2-hydroxy derivatives and 16 α -OHE1 have opposite biological properties. Cell proliferative activity of the 2-hydroxy metabolites is nil, while 16 α -OHE1 is a powerful estrogen agonist. The 4-hydroxy derivatives are also estrogen agonists, but their relative concentrations are smaller, so they may have less impact on cancer risk compared to the more abundant 2- and 16 α -derivatives. The carcinogenic

affects of 4-OHE1 may be due to the effects of toxic quinone metabolites rather than to estrogen agonist effects.¹⁹ Note that the 16 α -derivative of estrone is the precursor to relatively inactive estriol. Apparently, it is the unique orientation of the 16 α -OH group with the keto group of estrone that leads to the potent effects of this metabolite.²⁰ Sixteen- α -OHE1 can bind covalently to sites in the endoplasmic reticulum while becoming simultaneously bound to nuclear estrogen receptor sites.²¹ This binding stimulates heightened activity for days instead of hours. The 16 α -OHE1 effects persist until the binding proteins are degraded. Such increased cell proliferative and genotoxic effects appear to be a mechanism of cancer induction by tumor viruses, carcinogens, and oncogenes associated with breast cancer.²²

Early evidence suggested that 2-OHE1, at most, behaved as a weak estrogen and probably more as an anti-estrogen in several models.^{23,24} Cell culture studies in subsequent years also identified 2-OHE1 as a weak promotional estrogen that was less potent than 16 α -OHE1 at initiating cell proliferation.²⁵ Comparison of the relative potencies of 2-OHE1 and 16 α -OHE1 at transforming cells showed 16 α -OHE1 exhibited increased unscheduled DNA synthesis, proliferation, and anchorage-independent growth relative to 2-OHE1, which showed less activity than estradiol in each of these parameters.^{26,27} In long-term proliferation studies, persistent proliferation was observed after treatment of human ER-positive cancer cells with 16 α -OHE1 but not with 2-OHE1.²⁸

A prospective study examined estrogen metabolite ratios in 5,104 women age 35 and older where the median follow-up time to diagnosis was 9.5 years.²⁹ The researchers used the calculated ratio of 2-OHE1 to 16 α -OHE1 (2/16 α ratio) as a biomarker. Subjects with metabolite ratios in the highest tertile appeared to show a trend toward lower risk of breast cancer relative to those in the lowest tertile, although the participant size was not large enough to reach statistical significance.

Figure 2. *Hydroxy Derivatives of Estrone*



Postmenopausal women at baseline who went on to develop breast cancer showed a statistically significant 15-percent lower ratio than matched control subjects. A more recent prospective study of 10,876 Italian women age 35-69 years, who were followed for an average of 5.5 years, examined 144 breast cancer cases and four matched controls for each case. Among the group of premenopausal women, a higher 2/16 α ratio at baseline was correlated with a reduced risk of breast cancer. When the results of this cohort were divided into quintiles, women in the highest ratio quintile had the lowest risk.³⁰

Variations of the 2/16 α ratio between actual breast cancer cases and controls have been examined.³¹ Postmenopausal women with breast cancer have lower ratios than their matched controls. These results led the investigators to suggest a strong inverse association of the 2/16 α ratio and a strong positive association of 16 α -OHE1

with breast cancer in this subset of women. At about the time these studies were demonstrating a strong statistical association between the 2/16 α ratio and breast cancer, other groups were clarifying the potential causal relationship between increased 16 α -OHE1 and low 2-OHE1. A bifunctional pathway involving both estrogen receptor activation of protein transcription and direct genotoxic DNA alterations was proposed.²⁰ Previously, direct genotoxic effects had been measured by observing increases in proliferative activity and DNA repair in mammary epithelial cell lines.²⁶ In 1982 a 50-percent increase in 16 α -hydroxylation among patients with breast cancer compared to controls was demonstrated.³² This increase in 16 α -hydroxylation has important disease promotion effects that may be seen at both the genotoxic and hormonal levels of cancer generation. Although the 1982 report showed no association between stage of breast cancer disease

Table 2a. *Estrogen Metabolite-Cancer Connections*

Year	Finding	Ref.
1982	16 α -hydroxylated estrogens are associated with breast cancer and suggested to have an etiological role.	32
1984	Women with breast or endometrial cancer have increased estrogen-16 α -hydroxylase activity.	33
1986	Daily excretion of urinary estrogen metabolites is quantified and total metabolite excretion is lowered by dietary fiber.	34
1988	16 α -OHE1 has a unique capacity to bind covalently and irreversibly with the endoplasmic reticulum.	21
1992	Evidence that both genotoxic damage and aberrant proliferation caused by 16 α -hydroxyestrone is found in mouse mammary cells.	26
1994	Agents that increase 2-OHE1 inhibit carcinogenesis.	35
1995	The ratio of 16- α OHE1 to 2-OHE1 is elevated in women and animals with high rates of mammary tumors	7
1995	Organochlorine pesticides activate CYP enzymes responsible for 16 α -hydroxestrone formation	17
1996	CYP1A1 polymorphism causes lower 2-OHE1/16 α -OHE1 ratios in African-American women compared to Caucasian women	36
1997	Exposure of mammary epithelial cells to 16 α -OHE1 results in genotoxic DNA damage and increased cell proliferation similar to that induced by the carcinogen 7,12-dimethyl-(α)benzanthracene (DMBA).	37
1997	A bifunctional pathway of genotoxic and estrogen receptor modulation is proposed for carcinogenesis of 16 α -OHE1.	20
1997	Data from women with breast cancer and age-matched controls shows a strong inverse association of the 2/16 α ratios with cancer.	31
1997	Favorable clinical outcomes are predicted for women with high 2/16 α ratios in 9.5 year follow up prospective study of 5104 women (Guernsey III study).	29
1998	Estradiol and 16 α -hydroxyestrone increase abnormal proliferation and malignant conversion of keratinocytes. The effect is blocked by I3C.	5
1998	Increase in ratio of urinary 2-OHE/16 α -OHE1 correlates with improvement in recurrent respiratory papillomatosis.	38

Table 2b. *Estrogen Metabolite-Cancer Connections*

Year	Finding	Ref.
1999	The ratio of 2OHE1 to 16 α -OHE1 does not differ between women who had been treated for breast cancer up to 7 years prior to measuring the levels compared to controls who had never used chemotherapeutic agents.	39
2000	A prospective study of 10,876 Italian women shows that women in the highest quintile of the 2/16 α ratio have risks approximately half that of the lowest quintile.	30
2001	Metabolism of estrogen to 16 α -OHE1 is required for enhancement of papillomavirus-induced apoptosis	4
2001	2/16 α - ratio is proposed as a biological marker for risk of head and neck cancer	40

and the 2/16 α ratio, later results demonstrated lower ratios in those patients with stages III and IV disease (compared with stages I and II), suggesting women with lower ratios may have a poorer prognosis.³¹ Table 2a and 2b outlines the relationships between estrogen metabolites and cancer risk.

In another case-control study,¹¹ a differential in estrone metabolites between women with breast cancer and control subjects was confirmed. This study revealed that in the cancer cases there was a significant decrease in production of 2-OHE1, while levels of 16 α -OHE1 remained unchanged. This evidence emphasizes the value of increasing 2-hydroxylation, even if 16-hydroxylation cannot be reduced. When the 2/16 α ratio was analyzed between the subsets of women, ratios for the control group were discovered to be equally distributed above and below 2.0, while the ratios for the case group were distributed such that 72 percent fell below 2.0, further suggesting a 2/16 α ratio under 2.0 may be associated with breast cancer.

Dietary Modification of Estrogen Metabolism

Insight regarding the mechanism of estrogen induction of cancer may be gained from an

understanding of estrogen metabolism. Simple dietary modification can induce significant improvement in estrogen metabolism, decreasing morbidity and mortality from cancer. With regard to dietary supplementation with the active food components of cruciferous vegetables, it must be emphasized that any powerful inducer of hepatic enzymes should be used with caution. Although no problems with human populations have been shown, there is some evidence of adverse effects of the compounds discussed below under some conditions.⁴¹⁻⁴³ The use of estrogen-metabolite testing to identify candidates and monitor progress should enhance the safe and effective use of these interventions.

Cruciferous Vegetables

The Cruciferae are any of a family of plants including cabbage, broccoli, turnip, and mustard. This food category is also referred to as Brassica, which is a large genus of Old World temperate zone herbs of the mustard family with beaked cylindrical pods. Other examples of the class are kale, rutabaga, Brussels sprouts, cauliflower, kohlrabi, and collard. They are rich dietary sources of indolylmethyl glucosinolate glucobrassicin that, on enzymatic hydrolysis, releases indole-3-carbinol (I-3-C).⁴⁴

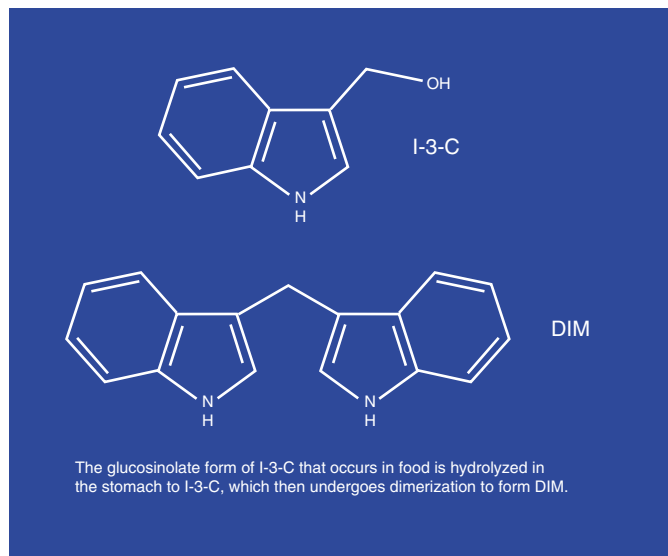
Table 3a. *Indole-3-Carbinol Modification of Estrogen Metabolites*

Year	Finding	Ref.
1978	I3C is shown to reduce the frequency of carcinogen-induced mammary tumors in rats by 75%.	47
1978	Dietary indoles inhibit aromatic hydrocarbon-induced neoplasia in mice stomach.	48
1986	I3C inhibits free radical-initiated hepatic oxidative damage in rat liver homogenates.	49
1987	I3C is a potent inducer of hepatic CYP450 and this mechanism is proposed for modification of xenobiotic metabolism.	46
1990	I3C strongly influences estradiol metabolism in humans by increasing 2-hydroxylation.	50
1991	1991 Mammary tumor incidence and latency in mice is reduced, hepatic CYP increased, and E2 2-hydroxylation increased 5-fold by dietary I3C supplements.	66
1993	I3C prevents human papillomavirus-induced tumors of the larynx or genital tract. Papilloma cyst formation in a mouse model falls from 100% to 25% in I3C-fed animals.	55
1994	In trout, anticarcinogenic effects are due to acid condensation products, not the parent compound, I3C.	56
1994	I3C has specific antigrowth effects on human breast cancer cells with little effect on estrogen non-responsive cells.	35
1994	I3C increases 2-hydroxylation in human cancer cell culture.	51
1994	Human subjects taking I3C at 400 mg/d for 3 months show sustained elevation of 2-OHE/16 α -OHE1 ratio with no detectable side effects.	67
1995	The naturally occurring phytochemical indole-3-carbinol prevents carcinogenic transformation in laboratory models of carcinogenesis.	17
1996	DIM suppresses human cancer cell growth by inducing apoptosis.	59
1997	Using the 2-OHE/16 α -OHE1 ELISA assay as the surrogate endpoint biomarker, an I3C minimum effective dose schedule of 300 mg/d is proposed for long-term breast cancer chemoprevention.	52
1997	Metabolism of cigarette smoke carcinogen is increased by dietary supplementation with I3C in mice.	57

Table 3b. *Indole-3-Carbinol Modification of Estrogen Metabolites*

Year	Finding	Ref.
1997	I3C effects in obese women are found to be similar to non-obese women, offering promise for reversal of the cancer-promoting effects of obesity.	63
1997	Studies of I3C effects on gene expression lead to new proposal of an antiproliferative pathway in human breast cancer cells.	60
1998	I3C blocks in vitro abnormal proliferation of premalignant keratinocyte induced by estradiol or 16 α -OHE1.	5
1998	Evidence for I3C to be safe and effective for treatment of recurrent respiratory papillomatosis in humans is found.	68
1999	Tamoxifen and I3C cooperate to arrest cell cycling in human breast cancer cells.	61
1999	Studies in mice indicate that I3C is a useful preventive for cervical-vaginal cancer and, possibly, other cancers with a papillomavirus component.	58
1997	Human mammary carcinoma-derived cells treated with I3C show 200% increase in quiescent/proliferative ratios, up to 18-fold increase in 2OHE1/16 α OHE1, a 2-fold increase in apoptosis, and a 60% inhibition in growth.	53
1999	Evidence of another I3C acidic conversion product, LTr-1, that inhibits both estrogen-dependent and -independent cancer cells as an antagonist of estrogen receptor function is found.	44
1999	I3C actions of inducing CYP1A1, increasing 2-hydroxylation, and inhibiting 4-hydroxylation of estrogens as well as direct effects of increasing apoptosis and blockage of cell cycling are reviewed.	54
1999	In a human cervical cancer cell line, I3C has anti-estrogenic activities that favor cancer prevention.	62
2000	Fifty percent of patients with cervical intraepithelial neoplasia had complete regression after oral I3C at 200 mg/d in a placebo-controlled trial where none of the controls have similar regression.	64
2000	Antitumor activities of I3C are associated with negative modulation of estrogen receptor transcription.	69
2000	Human breast cancer invasion and migration is suppressed by I3C via estrogen-dependent and -independent pathways.	70
2001	A nationwide population-based, case-control study in Sweden reveals an almost 2-fold reduction in relative risk for breast cancer when the highest decile is compared with the lowest in consumption of brassica.	65

Figure 3. Structures of Indole-3-Carbinol and Diindolylmethane



I-3-C undergoes dimerization in strong acid, like that normally present in gastric fluid. Several products may be formed, but the majority of ingested I-3-C is absorbed in the small intestine as the dimer, diindolylmethane (DIM) (Figure 3). Both I-3-C and DIM inhibit tumorigenesis. Multiple effects have been reported for the more extensively studied compound, I-3-C (Table 3a and 3b).

The use of I-3-C as a novel approach to breast cancer prevention was reviewed in 1995.⁴⁵ Hepatic CYP450 levels in the rat exhibit dose-response relationship to I-3-C.⁴⁵ The enzyme activities showed linear increases from 10 to 150 pmol/mg protein.min with oral doses ranging from 1 to 500 mmol/animal. When compared with other dietary indoles, I-3-C is the most potent inducer of 2-hydroxylation enzymes.

Associations of I-3-C with reduced incidence of tumors induced by dimethylbenzanthracene, aromatic hydrocarbons, or other free radical generators were the first signs of positive effects against cancer.⁴⁷⁻⁴⁹ Numerous lines of evidence support the conclusion that I-3-C induces hepatic CYP4501A1 with the resultant increase in 2-hydroxyestrogens.^{46,50-54} Several groups have

explored the question of I-3-C's impact on cell proliferative and neoplastic events. Data from animal models,⁵⁵⁻⁵⁸ human cell cultures,^{5,34,50,52,58-61} and human population cancer incidence^{52,63-65} have been examined (Table 3a and 3b).

Evidence for actions of I-3-C separate from that of DIM comes from studies of cell proliferation *in vitro*.⁵ A type of cell that represents premalignant keratinocytes showed abnormal proliferation when exposed to E2 or 16 α -OHE1. The proliferation was blocked by I-3-C. I-3-C induces cell cycle arrest in breast cancer cells through inhibition of cyclin-dependent gene expression.⁶⁰ So, although DIM appears to be the active I-3-C metabolite required for increased 2-hydroxylation, there may be other specific cell-regulating effects of I-3-C or its other metabolites not exhibited by DIM.

Evidence linking cigarette smoking with increased 2-OHE formation⁶³ adds support for use of I-3-C and DIM to reduce risk of hormone-dependent tumors by a similar mechanism. Female smokers have increased hepatic estrogen 2-hydroxylation, a finding that may account for the anti-estrogenic effects of cigarette smoking.⁷¹ The same metabolic effect is seen in men.⁷² Some of the components of cigarette smoke induce CYP1A1.

Responses to I-3-C vary among individuals due to genetic polymorphic variation in the inducibility of CYP1A1. The effect is easily seen in comparisons between Caucasian and African-American women. Baseline differences in the 2/16 α ratio were almost two-fold higher for Caucasian women (means of 2.25 vs. 1.42).³⁶ However, there were significant increases in 2/16 ratios in all subjects after ingestion of I-3-C, and African-American women had higher percentage increases than Caucasian women.⁷³ Women of both groups who carry Msp1 polymorphism in its heterozygous form respond much less to I-3-C. This observation means that women should be monitored to see if the ratio is increasing before continuing I-3-C supplementation. One specific dietary regimen that has been reported to be effective is a high fiber diet including the consumption of 50 g of cabbage or 100 g of broccoli twice weekly.¹⁰

Essential Fatty Acids

The omega-3 class of polyunsaturated fatty acids has emerged as another nutrient category with promise for adjunctive cancer prevention. Possible mechanisms for fatty acid chemoprotective effects include favoring series-3 eicosanoid synthesis and modulation of estrogen metabolism and estrogen-receptor binding. Increased 2-hydroxylation of estradiol occurs in human breast cancer cells grown with omega-3 fatty acids.⁷⁴ Additionally, docosahexaenoic acid (DHA) causes decreased binding of estradiol to the estrogen receptor.⁷⁵

With regard to cancer in estrogen-sensitive tissues, omega-6 fatty acids may have effects opposite those of the omega-3 series. High intake of omega-6 fatty acids linoleic acid and arachidonic acid inhibit the detoxification of estrogens by 2-hydroxylation⁷⁶ and increase 16 α -hydroxylation.⁷⁷

Omega-3 fatty acids have the potential for suppressing growth of estrogen-sensitive tumors. Studies cited above have shown that cells infected with papilloma viruses will respond favorably to increased 2-hydroxylation of estrone and estradiol with the converse effects seen in metabolism favoring 16 α -hydroxylation. DHA specifically inhibits the growth of cervical cells infected with human papillomavirus (HPV), while sparing growth inhibition in normal cells. Linoleic and eicosapentaenoic acids did not produce such effects.⁷⁸

Flax

Flaxseed is a rich source of plant lignans, a main class within the category of phytoestrogens. Flax lignans are metabolized via intestinal bacteria into enterolactone and enterodiol. Interestingly, these lignan derivatives have considerable structural similarity to estradiol, a mimicry which may explain the beneficial effect of flaxseed demonstrated on estrogen metabolism in several laboratories.

Flaxseed and its isolated lignans have been shown to display numerous chemoprotective effects *in vitro* and *in vivo*. Dietary intake of flaxseed has been found to reduce early markers of

risk for breast and colon cancer, and to inhibit breast tumor growth. The lignans within flaxseed are known to stimulate sex hormone binding globulin (SHBG) synthesis, inhibit aromatase activity, reduce breast tumor initiation, and inhibit growth of human breast tumor cells.⁷⁹ These lignans exert an indirect chemoprotective effect by helping remove endogenous estrogens via increased retention within the gut for elimination in the feces.^{79,80} Decreased enterohepatic circulation results in less presentation and/or availability of estrogens to target tissues, thus limiting the promotional effects of estrogens.

Regarding specific estrogen metabolism, flaxseed intake has been shown to positively influence estrogen metabolism by inducing 2-hydroxylation of estrone as well as to improve the ratio of 2/16 α -hydroxyestrone in postmenopausal women.⁷⁹ Dietary intake of 10 grams (1 Tbsp) of ground flaxseed per day for seven weeks produced the most dramatic effects in estrogen metabolism, while more moderate effects were observed at an intake of five grams daily. No change in levels of 16 α -OHE1 was observed in this study.

Soy

Epidemiological studies have linked soy consumption to a reduced risk for breast cancer.⁸¹ The connection between soy and breast cancer, however, has remained equivocal. Isoflavones are a class of phytoestrogens found in beans, legumes, soy, lentils, and other plant-based foods. Soy isoflavones, sometimes referred to as natural selective estrogen receptor modulators (SERMs), exhibit tissue specific responses due to interactions with estrogen receptors,⁸² inhibition of steroidogenic enzymes,⁸³ and interference with the binding of estrogen to SHBG.⁸⁴

Soy consumption has been shown to consistently reduce circulating levels of 17 β -estradiol in women.⁸⁵ Because an alteration in estrogen metabolism is a potential mechanism for this effect, excretion of the major estrogen metabolites has been examined in isoflavone-based studies. The results indicate soy isoflavones operate somewhat differently than via simple induction of CYP1A1. When women were maintained on an

Table 4. Association of Relative Risk Factors for Breast Cancer with 2/16 α Ratios

Report	2/16 α Ratio	Relative Risk
1 [30]	< 1.80	1.00
	1.80-2.30	0.76
	2.31-2.72	0.60
	2.73-3.29	0.62
	> 3.29	0.55
2 [31]		Odds Ratio
	> 1.91	1.00
	1.38-1.90	1.50
	< 1.38	1.95

isoflavone-rich diet for one complete menstrual cycle, their urinary excretion of 2-OHE1 increased by 47 percent and the 2/16 α ratio increased by 27 percent.⁸⁶

In other studies, decreased 16 α -OHE1, 4-OHE1, and 4-OHE2 were shown at soy isoflavone intakes of 130 mg/day, compared to low isoflavone diets (7-10 mg/day). An increase was seen in the 2/16 α ratio for both moderate and high isoflavone-containing diets, yet the moderate soy diet caused higher excretion levels of 2-OHE1 and a greater increase in the 2/16 α ratio than the high soy diet.^{87,88}

Soy isoflavones may have dual effects on breast cancer prevention: decreased effective circulating estrogen levels and increased ratios of anticarcinogenic/genotoxic metabolites in premenopausal women.⁸⁶ These effects have not been demonstrated for postmenopausal women. Also, there is still controversy over the total impact of

increased intake of soy products because of phytate effects on trace element status and changes induced by processing of soy protein. A definitive statement that soy reduces cancer risk cannot be made at this time, but there is considerable evidence of a protective effect on estrogen metabolism.⁸⁹

Measuring Estrogen Metabolites

The first attempts to estimate estrogen metabolites were by calculation based on excretion of non-catechol estrogens.⁹⁰ This calculation held true for patients with thyroid disorders, but not for the general patient population. Early radioisotope studies found that in normal subjects 30-40 percent of estradiol was converted to 2-hydroxy derivatives while 16 α -hydroxylation accounted for about 10-12 percent.⁹¹

Direct Measurements of Metabolites

An ELISA method is available for direct measurement of the sum of 2-OHE1 and 2-OHE2 metabolites and of 16 α -OHE1.⁹² This method has been validated against the gas chromatographic-mass spectrometric procedure⁹³ in which the metabolites are independently quantified.⁹⁴ With the improved ELISA method currently in use, a random specimen urinary metabolite ratio has been shown to represent an individual's 2-OHE1/16 α -OHE1 metabolite status. Urine was the specimen used in the studies of estrogen metabolite measurements cited in the tables and discussion above. Efforts to measure metabolites in serum with EIA methods have displayed large variations and lack of inter-laboratory agreement in reported concentrations.⁹⁵ The between-run coefficients of variation in a recent report was 30 percent and when these authors compared their data to a separate group using an RIA procedure, serum 2-OHE1

levels approximately four times greater were found.

For urine specimens a reference limit of ≥ 2.0 is generally used for the 2-OHE1/16 α -OHE1 ratio measured by the ELISA method. The laboratory report may show the individual concentrations of measured metabolites, but the critical parameter is the ratio that shows the protective relationship of the 2-hydroxylation pathway. People with 2/16 α ratio values below 2.0 should be advised of measures that can stimulate 2-hydroxylation. In the authors' laboratory, 52 percent of a general patient population was found to have ratio values below 2.0. Ratio values above 10 are sometimes found. Although there are no published data on effects of high ratios, 8.0 seems to be a reasonable limit for how high the value might be allowed to rise before advising moderation of inducement efforts, especially I-3-C or DIM supplementation. Bone formation is stimulated by 16 α -OHE1 in a manner similar to E2, so there is a potential for increased risk of osteoporosis for patients with very high 2/16 ratios, especially if the high ratio is caused by very low 16 α -OHE1.⁹⁶ Cancer risk reductions maximize at ratio values slightly above 2.0.

Specimen Timing

There are three important questions regarding the timing of specimen collection for urinary estrogen metabolite testing: (1) For premenopausal women, is there an ideal time during the menstrual cycle? (2) Does menstrual status affect the results of the assay? and (3) Does the timing of the urine collection throughout the day affect the result?

The effects of the menstrual cycle on urinary estrogen metabolites were studied in six healthy premenopausal women.⁹⁷ Early follicular, mid-follicular, peri-ovulatory, and mid-luteal 24-hour urine specimens were analyzed. While the concentrations of E1, E2, and the metabolites varied approximately five-fold from early follicular to peri-ovulatory specimens, the 2/16 α ratio varied by only 18 percent. Variations of the ratio with time of day for specimen gathering, and time of month for menstruating women seem to

be insignificant.⁹⁸ A further report found no significant differences in the mean 2/16 α ratio with the menstrual phase. It was also concluded there are no significant differences when the ratio is measured on 24-hour and first-morning urine or when multiple specimens are taken over a 24-hour period.⁹⁹ For best accuracy in measurement levels, most authorities agree the more concentrated first morning urine is best. The value of the 2/16 α ratio in a single urine sample reflects reasonably well an individual's level of the biomarker over a two-month period.¹⁰⁰ In a study of long-term variation, two spot morning urine samples were collected at an interval of one year from 87 postmenopausal women being monitored in the NHSII prospective cohort study. Eighty-three percent of the subjects maintained quartile status from one year to the next, leading to the conclusion that a single specimen can classify women into the appropriate quartile of risk relatively well.¹⁰¹

The observation that individual women maintain consistent patterns of monthly fluctuations in metabolites⁹⁹ means patients should be instructed to collect urine specimens at a consistent point of their menstrual cycle for initial and follow-up testing. An age-related decline has been reported in the urinary concentrations of metabolites; although no difference was observed between menstrual status and the 2/16 α ratio.

Calculations of Relative Risk

There is sufficient data from measurements of the 2/16 α ratios to derive values of relative risk of breast cancer in women. Two groups have reported relative risk categories based on case-control studies of women with a diagnosis of invasive breast cancer (Table 4). By either source, the risk is shown to nearly double when individuals with highest ratios are compared to those with lowest.

Conclusion

Approximately one-third of all cancer cases are related to dietary influences, and several lines of evidence indicate a female's diet during adolescence can affect her health during her mid-thirties. Whether a given woman has other risk

factors such as viral exposures or enzyme induction resistance, awareness of estrogen metabolism tendencies can be an important part of overall evaluation of cancer risk. The incidence of cancer of the breast and other estrogen-sensitive tissue is increased by exposure to estrogen and especially by increased 16α -OHE1 in the presence of low conversion to 2-OHE1. The 2/16 α ratio is the most modifiable and possibly the single greatest factor impacting estrogen-sensitive cancer risk. The effects of 16α -OHE1 elevations begin as soon as a female begins to secrete high levels of estrogens, so the earlier dietary interventions are begun, the greater the risk reduction.

Numerous studies have shown pre- and postmenopausal women with urinary 2/16 α ratios above 2.0 have reduced risk for estrogen-sensitive cancers. Particular candidates for testing include women identified to be at high risk due to family history or genetic testing, those with increased estrogen exposure over their lifetime, and women with a history of breast cancer eager to prevent recurrence. Since the development of cancer from a few malignant cells to a diagnosable tumor can have a long incubation period, perhaps years or decades, it is extremely valuable to offer women an objective method to assess risk for estrogen sensitive cancers as early as possible. The simple, non-invasive urine 2/16 α ratio test offers the promise of earlier determination of cancer risk, and simple, cost-effective, and non-toxic risk modification in the form of dietary and nutritional interventions.

Conclusive data has yet to be published showing cancer incidence falls when individuals with a low 2/16 α ratio are treated solely with interventions that raise the ratio by inducing CYP1A1. Therefore, those who consider using such treatments should proceed on the weight of the evidence presented to date. Further investigation is warranted, specifically long-term prospective studies that can yield definitive data regarding the magnitude of risk reduction. Progress may then be expected in national public education about dietary and lifestyle practices that can help assure favorable estrogen metabolism in all women.

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