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REVIEW ARTICLE

Estrogen Metabolism: A Complex Web

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ABSTRACT

An association between estrogen exposure and promotion of cancer is well established; however, much data also exists to support a beneficial role of estrogen in health. The paradox of estrogen's beneficial or detrimental nature has been attributed in part to excess estrogen, but the level of estrogen exposure alone does not explain the diverse research data on its effects. Estrogen metabolism is complex and results in several metabolites, many of which show estrogenic activity. Interest in estrogen metabolism has now focused on its hydroxylation by the cytochrome P450 enzymes, which leads primarily to the 16-hydroxy, 2-hydroxy, and 4-hydroxy estrogen metabolites. These metabolites appear to play differential roles: for example, data has suggested that the 16-hydroxy and/or 4-hydroxy estrogens may promote cell proliferation and tumorigenesis, whereas the 2-hydroxyestrogens appear to inhibit proliferation of cancer cells. The different activities of these metabolites may explain the diverse nature of estrogen. Furthermore, since the cytochrome P450 enzymes are responsible for generation of these metabolites, and these enzymes are highly responsive to diet, environmental, pharmacologic and lifestyle factors, understanding their influence on estrogen metabolism may help explain the association between dietary factors and a lower risk of hormone-dependent cancers.

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ESTROGEN AND CANCER

Estrogen has long been known to contribute to the promotion of several hormone-dependent cancers. A large and compelling body of evidence exists to implicate estrogen with risk of cancers of the breast and endometrium, and some data suggest an association may exist between estrogen and ovarian cancer as well.[1-4] Cancers of the breast, uterus, and ovaries are three of the top five cancers in women, and together account for an estimated 240,000 new cancer cases a year in the United States alone.[5] Research data also suggests an association between prostate cancer and estrogen; cancer of the prostate is the second leading cause of death from cancer in U.S. men.[3,6]

The observations that increased exposure to endogenous or exogenous estrogens is associated with an increased risk of breast and uterine cancers in both humans and animals are well documented.[1,4,7,8] *In vitro* data shows that prolonged estrogen exposure promotes cell proliferation, chromosomal irregularities, promotion of tumor growth, and DNA damage.[5,9,10] Human studies have found associations between hormone replacement therapy (HRT) and oral contraceptive use with an increased risk of breast cancer. Studies consistently show an increased risk of endometrial cancer with HRT as well — although data on oral contraceptive use and risks of endometrial and ovarian cancer are not so conclusive and suggest estrogen may promote a slight decrease in risk. Still, the animal data, mechanistic studies, and consistent associations seen between estrogen therapy in postmenopausal women and risks of breast and endometrial cancer are compelling. So strong are these observations that the Expert Panel of the National Institute of Environmental Health Science's National Toxicology Program (NTP) voted nearly unanimously to list steroidal estrogens on the 10th Edition Report on Carcinogens (RoC, 2002) as "known to be a human carcinogen".[11]

The implication of this toxic nature of estrogen is startling. After all, estrogen is a naturally occurring compound in both men and women and is necessary for health. The health benefits of estrogen have been reported in the literature almost as much as its toxic effects. For example, estrogen is important for the growth, differentiation, and function of female reproductive tissues, has a beneficial effect on bone formation and maintenance, has been implicated as a cardioprotective factor, and may decrease cognitive decline with age.[12-14] And yet, research data supports that it is also carcinogenic.

So, is estrogen good or bad? Healer, or harbinger of disease? How can one compound have so many diverse roles, be considered so damaging, and yet be so necessary for survival? The answer may lie in the fact that "estrogen" is not one compound, but instead describes a family of compounds with estrogenic activities, which include a number of estrogen metabolites. Recent research indicates that these metabolites may not only be active, but may engage in very different activities than the parent compound from which they are derived. How our body metabolizes estrogen may be as important, and potentially more relevant, to health benefits or disease risk than the amounts of endogenous and exogenous estrogens present. Moreover, estrogen metabolism involves the cytochrome P450 detoxification enzymes, and therefore is highly responsive to dietary, lifestyle, pharmacologic, and environmental factors, suggesting the risk of estrogen's adverse effects may also be modifiable.

ESTROGEN METABOLISM AND THE DETOXIFICATION ENZYMES

The term "estrogen" is used to collectively describe the female hormones, primarily composed of estrone (E1), estradiol (E2), and estriol (E3). In women with normal menstrual cycles, estrogens are synthesized from cholesterol in the ovaries in response to pituitary hormones. The ovarian follicle secretes 70 to 500 micrograms of E2 per day, depending on the phase of the menstrual cycle.[15] In men and in postmenopausal women, endogenous estrogen is produced in the peripheral tissues by the conversion of androstenedione, which is secreted by the adrenal cortex, to E1. The total estrogen produced after menopause, however, is far less than that produced during a woman's reproductive years.

The conversion of E1 to E2 is reversible, and both E1 and E2 can yield the same family of estrogen metabolites.[10,16] E2 is considerably more potent in classical assays for estrogenic activity than is E1, and is considered the most estrogenic of the compounds. Interconversion of E1 to E2 is performed by the 17β-hydroxysteroid dehydrogenase, which is present not only in steroidogenic tissues, but in many peripheral tissues, and its relative activity is considered one factor in the regulation of estrogen activity in tissues. E3 is synthesized primarily in the placenta and is the primary estrogen during pregnancy, but is not a major active estrogen in the male or non-pregnant female.

Over 30 different metabolites of E1 or E2 have been isolated *in vitro*; however, the primary metabolites *in vivo* appear to result from hydroxylation at three specific sites on the estrogen backbone, the 2-carbon, the 16-carbon, and the 4-carbon, yielding 2-hydroxyestrone or 2-hydroxyestradiol (2OHE), 16α-hydroxyestrone or 16α-hydroxyestradiol (16OHE), and 4-hydroxyestrone or 4-hydroxyestradiol (4OHE), respectively. The metabolic pathways are schematically represented in Figure 1.

Cytochrome P450 enzymes are responsible for hydroxylation of E1 and E2 to their respective metabolites, and different cytochrome P450s modulate different hydroxylations. Although there is overlap in specificity of the hydroxylation, in general CYP1A1/1A2 are the major enzymes involved in providing the 2-hydroxylated estrogen metabolites in humans; other P450s, such as the CYP2B1/2B2, 2C6, and the 3A family, contribute to 2OHE generation at low levels. The 16α-hydroxylation appears to be primarily produced by the CYP3A4/5 enzyme in humans. The 4-hydroxylation pathway appears to be a minor pathway in humans but, as described below, may be particularly important in the relationship between cancer and estrogen metabolism. Current data suggests the CYP1B1, and possibly some of the 3A enzymes, are the major contributors of 4-hydroxylated estrogens.[10,17]

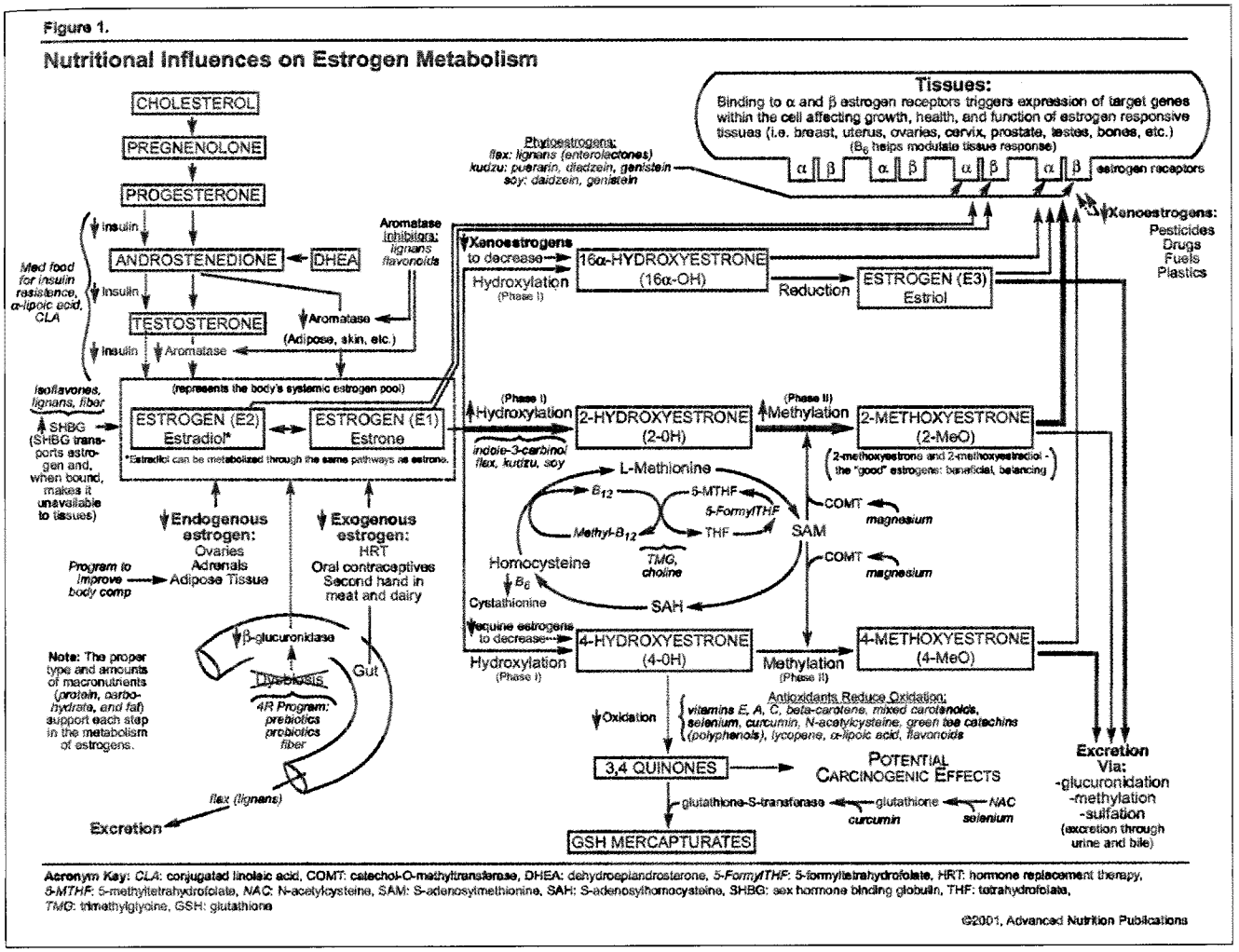
The 2OHE and 4OHE metabolites are collectively called the catecholestrogens, and can be further metabolized by methylation. When these catecholestrogens are not methylated, they can be readily oxidized to quinones, which are highly reactive and can damage DNA and promote carcinogenesis directly or indirectly through the generation of reactive oxygen species. Glutathione-S-transferase can convert the estrogen quinone metabolites to inactive mercapturates, which can be excreted. Antioxidants can quench the damaging reactive oxygen species that may be generated by these quinones; however, a more effective way of minimizing potential damage is by methylation of the catecholestrogens.

The catecholestrogens are methylated by the catechol-*O*-methyltransferase (COMT) enzyme, which is the same enzyme responsible for methylation of catecholamines and intermediates of melanin.[10,18,19] COMT is present in most tissues, and requires S-adenosylmethionine (SAM) and magnesium as cofactors.[20] Some evidence exists to suggest that high levels of catecholestrogens can compete for COMT activity with the catecholamines, resulting in inhibition of the production of neurotransmitters such as epinephrine, norepinephrine, and dopamine.[18] This observation suggests an interplay exists between neurotransmission and estrogen metabolism.

THE BENEFICIAL ESTROGEN METABOLITES: 2OHE AND ITS METHOXY DERIVATIVE

The 2OHE metabolites are capable of binding to the

Figure 1.



estrogen receptors; however, they appear to have very weak estrogenic activity. In contrast to the other estrogen family members, 2OHE has been shown to partially antagonize the growth stimulatory effect of E2 in cultured human MCF-7 breast cancer cells.[10] Furthermore, 2OHE can induce apoptosis and thereby inhibit cell proliferation, an important mechanism in the prevention of cancer.[21]

2OHE is formed mainly in the liver, but can also be generated directly in target tissues such as uterus and breast. It is the major catecholestrogen found in urine; however, little 2OHE is found in circulation because free 2OHE appears to be rapidly converted to its methoxy derivative, 2MeOE. 2OHE derivatives are also tight binders of sex-hormone binding globulin (SHBG) and, therefore, are primarily found bound to SHBG in serum.

Recent data suggests that 2MeOE manifests beneficial properties on its own; for instance, it has been shown to inhibit breast cancer cells and adipocyte proliferation.[22,23] *In vitro* studies have shown that 2MeOE inhibits angiogenesis, possibly by promoting apoptosis of endothelial cells, and it may function similarly to paclitaxel on stabilizing microtubules.[21,24] These observations support that the 2-hydroxylated estrogens and their methoxy derivatives are anticarcinogenic, which is why these metabolites are generally termed the "good" or beneficial estrogens in the literature.

CANCER AND ESTROGEN METABOLITES

While little controversy surrounds the notion that 2OHE and its derivatives are the most beneficial, or "safe", estrogen metabolites, much controversy exists as to which of the other metabolites is the primary culprit in cancer promotion. 16OHE and 4OHE are the primary estrogen metabolites associated with genotoxic effects.[19,25-27] 16OHE has been shown to promote proliferation of breast tissue; increases in both cell proliferation and DNA damage have been noted in cell culture studies as well as in animals treated with 16OHE.[19,28-30] The levels of 16OHE have been shown to be increased in mouse strains with a high incidence of breast cancer in a manner in which the increase parallels the degree of the cancer.[8] Higher levels of 16OHE

have also been seen in women with breast cancer as compared to those who do not have the cancer.[8]

Although 16OHE appears to be associated with cancer, it is also an active estrogen in the body with a beneficial role to play, and may be the main estrogen metabolite to promote bone health. Some researchers argue that 16OHE is not responsible for estrogen's genotoxicity, and suggest instead that it is the catecholestrogens, in particular the minor metabolite 4OHE, that is to blame. 4OHE is similar to E2 in its ability to bind and activate the estrogen receptor; however, data suggests this binding carries a reduced dissociation rate compared to E2, indicating that 4OHE may bind more tightly and be more estrogenic overall.[10] Administration of 4OHE to animals alters secretion of luteinizing hormone and follicle stimulating hormone, leads to more induction of progesterone receptor than the same amount of administered E2, and supports embryo implantation. 4OHE appears to stimulate the arachidonic acid pathway as well, and it has been suggested that through this mechanism 4OHE may be the estrogenic metabolite that modulates the physiological effects of prostaglandins.

Probably the strongest biochemical mechanism to explain estrogen's genotoxic effect comes from data on the reactive quinones from the catecholestrogens. As mentioned previously, the catecholestrogens can be readily converted to reactive quinones that may induce DNA damage directly. These quinones may also cause DNA mutations as a result of redox cycling between the quinone and its semiquinone radical, which generates reactive oxygen species including superoxide, hydrogen peroxide, and hydroxyl radicals.[10,19,27,31] Excessive production of reactive oxygen species has been reported in breast cancer tissue, and free-radical toxicity, which manifests as DNA single-strand breaks, lipid peroxidation, and chromosomal abnormalities, has been reported in hamsters treated with E2.[31]

Although the ability to generate free radicals provides a strong mechanism for explaining the carcinogenic effect of estrogen, it is controversial. One reason for this controversy is that 2OHE can also form a quinone, and yet this metabolic pathway appears to be protective. Some researchers argue that this is because 2OHE is readily methylated to a safer estrogen metabolite, which cannot form the quinone, whereas 4OHE is not quickly methylated. For example, 2OHE inhibits COMT methylation of 4OHE, whereas 4OHE does not inhibit methylation of 2OHE, suggesting a substrate preference of COMT for 2OHE.[10] In addition, 4OHE has been shown to be a strong carcinogen towards hamster kidney under conditions in which 2OHE is not carcinogenic.[10]

THE 2OHE TO 16OHE RATIO

It is unknown whether 16OHE or 4OHE is the primary genotoxic metabolite, and it is possible that each plays a role in different cancers at different times. However, a consensus appears to be forming that the ability of a person to generate sufficient levels of 2OHE over these other metabolites is crucial to health promotion. Some of these observations have been based on analysis of the 2OHE to 16OHE ratio in urine and/or serum. Michnovicz and Bradlow [32] are credited with the initial observation of an increased ratio of 2OHE to 16OHE in response to dietary factors such as vegetable consumption. Since vegetable consumption is associated with a decreased risk of many cancers, they proposed that this ratio is important in understanding the influence of diet on hormone-dependent cancers such as breast cancer. Subsequent observations in Oriental and Caucasian women, and in postmenopausal women with and without breast cancer, supported the hypothesis and showed that those groups with a lower risk of breast cancer appear to have a higher 2OHE to 16OHE ratio.[30,33]

A recent 5-year prospective study of 10,786 women was conducted to investigate the role of estrogen metabolism as a predictor of breast cancer, specifically the ratio of 2OHE to 16OHE.[26] In this study, premenopausal women who developed breast cancer had a decreased 2OHE to 16OHE ratio, resulting in a higher percentage of 16OHE than 2OHE. Women with predominately 2OHE were 40% less likely to have developed breast cancer during the 5 years. Another recent case-control study that began in 1977 found that postmenopausal women who developed breast cancer had a 15% lower 2OHE to 16OHE ratio than control subjects.[28] Furthermore, those with the highest 2OHE to 16OHE ratios had about a 30% lower risk to breast cancer than women with lower ratios.

Riza *et al.* [34] have recently published results of a study performed in Northern Greece in which the 2OHE to 16OHE excretion levels of 70 women with mammographic parenchymal patterns (P2/DY) were compared to 70 women with N1 mammographic patterns. In contrast to previous observations, these authors report that the ratio of 2OHE to 16OHE was higher in the women with P2/DY patterns than the women with N1 patterns. This study evaluated urinary excretion levels, and did not report serum levels, nor were the levels of methylated metabolites of the 4OHE derivative reported. In addition, the women in this study were found to have an overall higher body mass index than that reported for other Western populations.

The role of these estrogens in cancer promotion is far from clear; however, the majority of data continues to support the assertion that women who metabolize a larger proportion of their endogenous estrogen via the C-2 pathway may have a significantly lower risk of breast cancer. It is interesting to note that cyst fluid from women with fibrocystic breast disease has been found to contain the hydroxylated estrogens; in some instances this fluid has been shown to have a much higher amount of 16OHE, as well as higher levels of 4OHE than of 2OHE.[35,36]

ESTROGEN METABOLISM AND GENETIC UNIQUENESS

Using a combined analysis of twin registries from Sweden, Denmark, and Finland, heritable factors were estimated to account for 27% of risk for breast cancer, with the remaining due to shared and non-shared environmental factors.[37] Much of the genetic association of cancer has been attributed to the BRCA1 and BRCA2 genes, which account for up to 70% of all hereditary breast cancer cases.[38] Individuals with these mutations have a 50%-80% lifetime risk of developing breast cancer, and are more susceptible to ovarian cancer as well. These genes are not directly involved in estrogen metabolism, but function as tumor suppressor genes.

Many researchers have suggested that it may be possible to identify many gene mutations, or polymorphisms, that may individually contribute only slightly to increased risk of hormone-dependent diseases, but when combined with other gene polymorphisms can together contribute to increased risk in specific individuals. Primary genes in consideration by this polygenic model for disease risk include genes involved in estrogen synthesis and metabolism, such as aromatase (CYP19) and COMT, as well as the estrogen receptors.[39-41] For example, the protein encoded by a polymorphic CYP1B1 gene has been shown to convert estrogen to the 4OHE metabolite more efficiently than the wild type protein *in vitro*.[42] Nested case control analysis of data from the Nurse's Health Study cohort showed only a modest, but not significant, association between the polymorphisms and plasma estrogen levels.[43] Polymorphisms in the COMT gene have also shown modest associations with cancer susceptibility.[44,45] In a recent analysis of 483 Finnish breast cancer cases and 482 population controls, the combined effect of polymorphisms in COMT and in the phase II detoxification enzyme glutathione S-transferase (GST) showed no increase in overall breast cancer risk, but a substantially increased risk of breast cancer was seen in women who had combinations of these polymorphic genes and were on HRT.[46]

If this polygenic model is correct, then the influence of individual polymorphisms on estrogen metabolism, and the subsequent risk to hormone-dependent diseases, will be small and likely non-significant when studied individually. Instead, as suggested by the studies with COMT and GST, combinatorial analyses will be required. In addition, if diet, environment, and lifestyle influence the expression of some, or all, of these polymorphisms, then the risk for each individual would be dependent on the combination of genetics and environment and would be very individualized. Willett addressed this issue in a recent review of genomics and disease prevention, in which he questions whether this type of data will contribute more than the currently available measurements of serum estrogen or estrogen metabolites.[47] One consideration, however, is the potential for early intervention once these genetic associations are established, in which intervention could proceed prior to any increase in metabolites with their subsequent deleterious effects.

Table 1. Mechanisms through which nutritional factors may influence estrogen metabolism

Mechanism of Action	Phytonutrient
Promote C-2 hydroxylation over C-4 and/or C-16α hydroxylation of estrogens	Cruciferous vegetables, indole-3-carbinol, isoflavones (e. g. , soy, kudzu)
May reduce the oxidation of catecholestrogens (2-OH and 4-OH) to their reactive quinones	Vitamins E and C, curcumin, green tea catechins, lycopene, flavonoids
Support the methylation of catecholestrogens	Folate, vitamins B_2, B_6 and B_{12}, trimethylglycine, magnesium
Increase circulating concentrations of sex hormone binding globulin (SHBG), thus reducing levels of unbound, active estrogens	Fiber, lignans (flaxseed), isoflavones (e.g., soy)
Inhibit the activity of aromatase, which converts testosterone and androstenedione into estradiol and estrone, respectively	Lignans (flaxseed), flavonoids
Promote the elimination of estrogens by inducing and/or supporting the activity of phase II detoxification enzymes	Turmeric (curcumin), d-limonene, glutathione, N-acetylcysteine
Reduce the activity of beta-glucuronidase, which deconjugates estrogens in the large intestine allowing them to be reabsorbed and re-metabolized	Fiber, probiotics (acidophilus, bifidobacteria), calcium-d-glucurate
Effect estrogen receptor binding and/or act as a selective estrogen receptor modifier (SERM)	Isoflavones (e.g., soy, kudzu, clover), lignans (flaxseed), vitamin B_6, indole-3-carbinol

NUTRITIONAL MODULATION OF ESTROGEN METABOLISM AND ACTIVITY

Although the role of genetics in estrogen metabolism is still unclear, it is known that multiple dietary and nutritional factors have the ability to influence estrogen synthesis and receptor activity, as well as the detoxification pathways through which estrogens are metabolized (Table 1, Figure 1). For example, there are many lifestyle factors that can influence the body's production of estrogen. Obesity increases endogenous estrogen production by fat tissue, where the enzyme aromatase converts adrenal hormones into estrogen.[1] Excess insulin in the bloodstream prompts the ovaries to secrete excess testosterone and reduces SHBG levels, thus increasing levels of free estrogen.[48] Alcohol consumption increases estrogen levels, and epidemiological studies suggest that moderate alcohol consumption increases the risk of breast cancer, an effect that may be synergistically enhanced when combined with estrogen replacement therapy.[49,50]

Much has been written about environmental estrogenic compounds, such as the isoflavones in soy and clover, or the lignans in flax. Over 360 plants have been characterized as having compounds with estrogenic activity. Animal products such as milk and meat may contain the actual estrogens themselves; in some cases this is from the use of estrogens in the animals. A recent review in this journal discussed the effect of xenoestrogens, such as chlorinated pesticides and estrogens in meat, on breast cancer.[51]

Less has been written about the influence of environmental compounds on estrogen metabolism itself; however, this may be the most profound impact of environment on hormone-dependent cancers. Diverse factors can add to the hormonal risk by decreasing the 2OHE to 16OHE ratio, including numerous pesticides and carcinogens, certain drugs such as cyclosporin and cimetidine, obesity, and genetic predisposition.[52-55] Specific phytonutrients have also been shown to significantly promote C-2 hydroxylation and increase the 2OHE to 16OHE ratio. Of these, one of the largest bodies of literature exists on the role of cruciferous vegetables in altering estrogen metabolism.

CRUCIFEROUS VEGETABLES, INDOLE-3-CARBINOL, AND ESTROGEN METABOLISM

Numerous epidemiological studies have shown that consumption of crucifers, such as broccoli, cabbage, and cauliflower, are associated with protection from several types of cancers.[56,57] Cruciferous vegetables contain glucosinolates that, when consumed, are hydrolyzed to several compounds with bioactivity, including indole-3-carbinol (I3C), crambene, iberin, and phenethylisothiocyanate. Although all of these compounds may have activity, recent animal investigations suggest the most bioactive with respect to the detoxification enzymes systems is I3C.[58-62]

Studies in human clinical trials have shown that I3C at doses of 400 to 500 mg per day can influence estrogen metabolism and promote formation of the protective estrogen 2OHE.[63-65] In a recent clinical trial, men and women supplemented with 500 mg and 400 mg of I3C, respectively, showed a significantly increased urinary excretion of 2OHE and a decrease in excretion of nearly all other metabolites, including E2 and 16OHE.[64] In a double-blind, placebo-controlled study of 57 women at increased risk for breast cancer, supplementation with I3C (300-400 mg/d for 4 weeks) resulted in an increased 2OHE:16OHE ratio.[66] Furthermore, early clinical trial data suggests that 200 mg and 400 mg per day doses of I3C may support remission in cervical intraepithelial neoplasia.[67] These data for I3C are compelling enough that the National Cancer Institute has nominated I3C for testing as a preventative for breast cancer.[68]

The mechanism by which I3C promotes 2OHE formation appears to involve the selective induction of the cytochrome P450 enzymes CYP 1A1 and 1A2, which facilitate the 2-hydroxylation of estrogens. Through this metabolic role, I3C promotes an increased ratio of 2OHE to 16OHE and may improve estrogen metabolism in women with poor diets or impaired detoxification.[25,63,64] I3C may also reduce the activity of the enzyme required for generation of the potentially carcinogenic 4OHE.[69]

Along with the ability to induce production of 2OHE by modifying phase I detoxification enzymes, I3C has been shown to induce phase II detoxification activities, regulate cell-cycle progression, suppress activation of invasion-promoting molecules associated with breast cancer cell metastasis, affect transcription of the estrogen receptors in human tumor cells, and reverse the activation of multidrug resistance in cancer cells.[70-73] Many of these activities can be related to the multiple actives produced by ingestion of I3C. Once in the stomach, it is converted to many active compounds, including diindolylmethane (DIM); indolo(3,2-b)carbazole (ICZ), which exhibits antiestrogenic activity and supports phase I detoxification activities; and hexahydrocyclonatriindole (HNTI), which binds the estrogen receptor and shows chemical structure similarities to tamoxifen.[34,74,75]

METHYLATION COFACTORS: VITAMIN B12 AND FOLATE

The B vitamins, such as B_6, B_{12}, and folate, function as important cofactors for enzymes involved in estrogen conjugation and methylation. In particular, these vitamins support maintenance of SAM, a required cofactor for methylation of the catecholestrogens by COMT. Therefore, decreased levels of these B vitamins may disrupt estrogen detoxification and lead to increased levels of circulating estrogens. Interestingly, several studies have reported associations of a COMT polymorphism, which results in a 3- to

4-fold decrease in COMT activity, with increased risk of breast cancer. A recent report furthering these observations found that a high homocysteine level and/or low folate status in combination with the presence of a low activity polymorphism in the COMT gene was associated with an increased risk of breast cancer.[76] Low serum vitamin B_{12} level has also been found associated with a higher incidence of breast cancer.[77]

B vitamins also play a role in the prevention of cancer because they are crucial for DNA synthesis and repair as well as the process of DNA methylation, which is essential for DNA stability and integrity and is an important regulator of gene expression. It has been shown that breast cancer cells have sites of hypomethylation at the estrogen receptor loci; however, the role of hypomethylation *in vivo* and the specificity of hypomethylation have not been fully explored.[10]

MAGNESIUM AND ESTROGEN METABOLISM

Magnesium is an essential cofactor for the COMT enzyme, and therefore optimizes the methylation and excretion of catecholestrogens.[31] Magnesium also promotes estrogen detoxification by directly increasing the activity of glucuronosyl transferase, an enzyme involved in hepatic glucuronidation. Ovarian hormones influence magnesium levels, triggering decreases at certain times during the menstrual cycle as well as altering the calcium to magnesium ratio. These cyclical changes may produce many of the well-known symptoms of premenstrual syndrome (PMS) in women who are deficient in magnesium and/or calcium.[78]

VITAMIN B_6 AND THE ESTROGEN RECEPTORS

Although data between vitamin B_6 and estrogen-dependent cancers has not been reported, it is worth noting that cell culture studies suggest vitamin B_6 may play a role in modulating estrogen activity via the estrogen receptor. Estrogens, like all steroid hormones, have a wide range of actions and affect almost all systems in the body, yet act in a tissue-specific manner. Estrogens act by binding with high affinity to the estrogen receptors in target cells. Once bound by estrogens, the estrogen-receptor complex undergoes a conformational change that promotes its binding to specific DNA sequences, sometimes referred to as HREs (hormone response elements). This transcription complex regulates the expression of target genes within a cell.[79,80]

It has been demonstrated that elevated intracellular concentrations of the active form of vitamin B_6 can lead to significantly decreased gene transcription responses when estrogen binds to the estrogen receptor.[81] By modulating estrogen-induced gene expression in this way, vitamin B_6 may attenuate the biological effects of estrogen, as well as functioning in support of methylation.

ISOFLAVONES AND LIGNANS

Isoflavones and lignans, also called phytoestrogens, are plant compounds currently being extensively investigated as a potential alternative therapy for a range of conditions associated with estrogen imbalance including menopausal symptoms, PMS, endometriosis, prevention of breast and prostate cancer, and protection against cardiovascular disease and osteoporosis.[79-83] The lignans and isoflavones have a similar structure to E2 and can bind to the estrogen receptors, which may account for much of their activity.

Recent research suggests that isoflavones may shift estrogen metabolism away from the C-16 pathway to the C-2 pathway, resulting in an increase in the 2OHE to 16OHE ratio in both pre- and postmenopausal women.[84,85] Soy is perhaps the most common food source of isoflavones, but others include legumes, alfalfa, clover, licorice root, and kudzu root. Biologically active isoflavones include genistein, daidzein, equol, and puerarin, and some of their plant precursors include formononetin, biochanins, genistin, and daidzin. Higher intakes of soy products and isoflavones, such as consumed in traditional Japanese diets, are associated with low rates of hormone-dependent cancers.[86] The average daily isoflavone intake of Japanese women is 20 to 80 mg, while that of American women is 1 to 3 mg.[87]

Lignans, which are found in fiber-rich foods, stimulate the production of SHBG in the liver, and therefore reduce the levels of free estrogen in circulation. They also inhibit aromatase activity, and may thereby decrease the conversion of testosterone and androstenedione into estrogens in fat and breast cells.[88] Lignans also have been shown to inhibit estrogen-sensitive breast cancer cell proliferation.[89] An influence of lignans on estrogen metabolism, in particular on the 2OHE to 16OHE ratio, has not been reported.

OTHER NUTRIENTS AND PHYTONUTRIENTS THAT MAY INFLUENCE ESTROGEN DETOXIFICATION

Other nutrients and phytonutrients are being investigated for their role in breast cancer prevention, and some data suggests these factors may influence detoxification of the estrogen metabolites. Curcumin, a polyphenol complex from the spice turmeric, has shown synergy with the isoflavone genistein in reducing xenoestrogen-induced growth of ER-positive and ER-negative breast cancer cells.[90] Curcumin also increases hepatic levels of glutathione and induces glutathione-S-transferase (GST) and glucuronosyl transferase, important in the phase II detoxification of quinones produced from the oxidation of catechol estrogens.[91,92] D-limonene, a naturally occurring monoterpene found in the oils of citrus fruits, promotes the detoxification of estrogen by inducing phase I and phase II enzymes in the liver, including GST, and has shown great promise in the prevention and treatment of breast and other cancers.[93,94]

Much interest is focusing on the role of the hydroxylated derivatives of estrogen in cancer, but estrogens are also detoxified by glucuronidation and sulfation, and some data has suggested that the glucuronidation pathway may also be important in protection from the genotoxic effects of estrogen metabolites. Glucuronic acid is conjugated with the estrogen to facilitate its elimination from the body; however, some intestinal bacteria possess an enzyme, β-glucuronidase, that uncouples the bond between excreted estrogen and glucuronic acid in the large intestine, allowing the estrogen to reenter circulation (enterohepatic recirculation).[95] Calcium-d-glucurate has been shown to inhibit proliferation of mammary tumor cells in culture, and to lower estradiol levels and inhibit the progression of cancer in animals.[95,96]

CONCLUSIONS

An abundance of evidence supports a role of estrogen in the development of cancer in tissues such as the breast, endometrium, ovary, uterus, and prostate. Estrogen is important for health, and so this connection with disease is confounding. The recent elucidation of the complex metabolism of estrogens *in vivo*, and the understanding that not just the parent estrogens — estrone, estriol, and estradiol – but, also their metabolites can support estrogenic activities is helping to clarify why estrogens can be associated with so many diverse effects. Furthermore, hormonal imbalances between progesterone, testosterone, and estrogen can lead to symptoms and conditions of estrogen dominance, which include PMS, endometriosis, uterine fibroid tumors, fibrocystic or painful breasts, cervical dysplasia, and systemic lupus erythematosis.

Fortunately, beneficial modulation of estrogen metabolism can be accomplished through modification of the diet, and by supplementation with select nutrients. A weight management program may also be very helpful in both reducing adipose aromatase activity and facilitating more desirable estrogen metabolism and excretion. The promotion of healthy estrogen metabolism may have profound significance for diseases and conditions in which these hormones play a role.

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