

ESTROGEN METABOLITES

Urinary Estrogen Metabolites & Their Relevance To Breast Cancer Risk

Much research has been done over the past 20-30 years to increase our understanding of the relationship between estrogens and their metabolites and the risk of breast and other estrogen-sensitive cancers. What has emerged, and is now universally accepted by the majority of scientists, is that high levels of 4-catechol estrogens (estradiol and estrone) and to a lesser extent the 2-catechol estrogens, and decreased methylation of these catechol estrogens, are associated with increased breast cancer risk. Catechol estrogen formation is one of the pathways whereby estradiol and estrone are metabolically prepped for elimination in the urine and feces. In a healthy individual, these transition catechol estrogens are constantly being formed and inactivated by methylation via catechol-O-methyltransferase (COMT), an enzyme present in the surface membranes of all cells. The catechol estrogens and their methylated forms are then subjected to sulfation and glucuronidation, which increases their solubility in the bloodstream, clearance rate, and elimination in urine. Methylated catechol estrogens are eliminated primarily via the urine, where they can be measured by GC or LC mass spectrometry.

Estrogens are prepared for elimination after they have interacted with cellular estrogen receptors in estrogen-sensitive tissues throughout the body (e.g. uterus, breasts, brain, skin, and bone) and modified genome expression in a well-controlled and beneficial way. Estradiol, the most potent of the three primary estrogens (estradiol, estrone, and estriol), plays an essential role in maintaining the health of nearly every tissue in the body, in particular the reproductive tissues, brain, skin, bone,

liver, and cardiovascular system. Physiological levels of estradiol formed cyclically with natural progesterone throughout a woman's premenopausal years maintain the health and youthfulness of these tissues. Menopause results in the loss of ovarian estrogen production and a consequent drop in circulating levels of estradiol. If, during menopause, estradiol drops well below the lower end seen in premenopausal women, this can be associated with adverse effects in the reproductive tissues (incontinence, vaginal dryness), brain (lowered neurotransmitters, increased hot flashes and night sweats), skin (more rapid aging), bone (accelerated loss and greater risk for osteoporosis and fracture), liver (compromised hormone metabolism and reduced synthesis of hormone binding globulins, reducing the circulating half-lives of hormones that are bound to them), and cardiovascular system (increased risk for insulin resistance, diabetes, and cardiovascular disease). Needless to say, estrogens, and specifically estradiol, are essential for maintaining health in both premenopausal and postmenopausal women. In this regard, estrogens could be considered the "Angel of Life" (1).

While estradiol plays this significant role in maintaining health, it can also have the opposite effect when certain catechol metabolites are formed in excess and not eliminated properly. Two separate enzymes are involved in converting estradiol and estrone to their respective 2- and 4-catechol derivatives. 2-catechol estrogens are formed by the interaction of the cytochrome enzyme designated CYP1A1. 4-catechol estrogens are formed from CYP1B1. If methylated, both 2- and 4-catechols are essentially

rendered inert (harmless), and are excreted in urine. In a healthy individual the formation of estrogens, their beneficial utilization by tissues, and their subsequent elimination in urine and feces are biochemically well coordinated and balanced. However, estradiol and estrone can turn bad in the same way as beneficial oils can go rancid. In fact, the process is very similar and involves oxidation of the estrogens to highly reactive and potentially dangerous metabolites. If these metabolites are not properly channeled down safe pathways of elimination, this could be damaging to some tissues in a way that may eventually be expressed as cancer, most notably breast cancer.

In the presence of excessive reactive oxygen species (ROS) such as peroxy lipids, formed mostly from trans-fats consumed in the diet, the catechol estrogens are further co-oxidized to highly reactive 2- and 4-estrogen quinones. In fact, the reason for avoiding trans-fats to protect the cardiovascular system against damage is the same reason to avoid them to protect the breasts from damage that may lead to breast cancer. ROS such as peroxy lipids are very electrophilic (electron hungry) molecules that, under normal circumstances, are inactivated rapidly by interaction with glutathione (the most abundant nucleophilic molecule in tissues, which donates electrons to reactive electrophiles to inactivate them) and glutathione transferase. However, if ROS are in abundance and glutathione levels are low, the highly reactive catechol quinones can bind to DNA, causing mutations that can lead to cancer. 4-quinone estrogens are considered much more dangerous than 2-quinone estrogens because the former cause DNA damage that leads to more permanent (unfixable) mutations (2) that can produce aberrant cancer cells, and given the right circumstances (e.g., a compromised immune system) eventually a breast tumor.

Thus, the degree of damage rendered by estrone or estradiol will depend on the following factors:

- 1) how much of the estrogen is present;
- 2) if the metabolism is predominantly down the 2- or 4-catechol estrogen pathway;

- 3) if the 16-hydroxyestrone/estriol metabolism pathway dominates over the 2- or 4-catechol estrogen pathway;
- 4) if adequate methylation pathways are present to help remove 2- and 4-catechol estrogens before they are converted to the more toxic estrogen quinones;
- 5) if adequate glutathione is present to react with estrogen quinones.

To understand better how estrogen metabolites are associated with risk for breast cancer, the urinary estrogen metabolites are evaluated based on the levels of the three parent estrogens (estradiol, estrone, estriol) and their metabolites. These include the estriol precursor 16-hydroxyestrone, the 2- and 4-hydroxylated metabolites of estradiol and estrone, and the 2- and 4-methylated metabolites of estradiol and estrone.

Nearly all research studies on catechol estrogens and breast cancer have concluded that in women not supplementing with exogenous estrogens, high levels of the 4-hydroxylated catechol estrogens (estradiol and estrone), and to a lesser extent 2-hydroxylated catechol estrogens, are associated with increased breast cancer risk (2-5).

While it is clear that high levels of 4-hydroxylated catechol estrogens are associated with increased risk, controversy persists regarding the significance of 16-hydroxyestrone, and its relationship to 2-hydroxyestrone, i.e. the 2/16 ratio. Previous research using mostly Enzyme Immuno Assays (EIAs), as opposed to more accurate GC- or LC-MS/MS methodology, concluded that a low 2/16 ratio was associated with increased breast cancer risk. Based on these data it was concluded that increasing the level of 2-hydroxy catechol estrogens with higher dietary consumption of cruciferous vegetables, or supplementation with extracts of them, e.g., indole-3-carbinol (I3C) and diindolemethane (DIM), would increase the 2/16 ratio and decrease risk for breast cancer. However, this hypothesis has been challenged repeatedly (6) and found to be inaccurate. More

recent research (7) confirms that high levels of 16-hydroxyestrone, which usually results in a low 2/16 ratio, are indeed associated with a slight increase in breast cancer risk in premenopausal women, but, paradoxically, a lower risk in postmenopausal women. Therefore, interpretation of 16-hydroxyestrone results and the 2/16 ratio is dependent on menopausal status.

As mentioned, methylation is an important mechanism whereby the catechol estrogens are prevented from being further converted (oxidized) to more reactive and dangerous quinone estrogens. Methylation occurs through the action of COMT and requires adequate levels of vitamins B6, B12, and folate. The relative amounts of methylation are evaluated by the ratio of a relatively inert catechol estrogen (2-hydroxy estrone) and one of the most reactive and dangerous estrogen metabolites (4-hydroxy-estrone) to their methylated counterparts (2-methoxy estrone and 4-methoxy-estrone, respectively).

IMPORTANT NOTE: Research on breast cancer and catechol estrogens in premenopausal and postmenopausal women cannot be, at this time, extrapolated to women on any form of estrogen replacement therapy. The interpretation is strictly to be used only for those women who are not taking any form of exogenous hormone replacement therapy that directly or indirectly affects the levels of estrogen and estrogen metabolites (i.e. estrogens, androgens, or progestogens). No published literature is available on urinary estrogen metabolites and the risk of breast cancer in women using estrogen replacement therapy.

General Guide to Results Interpretation

Lower levels of estradiol and estrone, and higher relative levels of 16-hydroxyestrone and estriol are associated with lower breast cancer risk (8) in postmenopausal women not supplementing with exogenous estrogens (i.e., estrogen replacement therapy). However, this is also dependent on the level of catechol estrogens present, their relative levels (2 vs 4), and how well they are methylated. If estrogens (estradiol and estrone) are low, as are the catechol estrogens, this would

portend a lower risk for breast cancer, but possibly a higher risk for symptoms (e.g., hot flashes), conditions (e.g., bone loss), and diseases (e.g., cardiovascular disease) associated with estrogen deficiency. When estradiol, estrone, and estriol are low, the methylated estrogens would be expected to be low also because of low levels of precursor catechol estrogens. If, however, catechol estrogens are elevated, regardless of the estradiol or estrone level, and methylated estrogens are low, this could indicate higher risk. Keep in mind that the catechol estrogens are not dangerous per se, unless converted to more reactive quinone estrogens. Whether the quinone estrogens damage DNA, or are rapidly inactivated will depend on many factors that are modifiable through diet and nutritional supplements. Excessive dietary consumption of unhealthy trans-fats oxidizes catechol estrogens to more dangerous quinone estrogens, and if glutathione is not present in adequate amounts the quinone estrogens are more likely to damage DNA, and lead to mutations that could be eventually expressed as breast cancer.

Prevention Strategies

- 1) Prevention strategies begin with reducing the overall burden of excessive estradiol and estrone in the absence of diminished levels of progesterone, which often occurs in the early phases of menopause (perimenopause-ages 35-55). Progesterone supplementation helps reduce the estrogen burden by increasing the conversion of E2 to E1 (activates 17-beta-hydroxysteroid dehydrogenase), and E1 to E1-sulfate (E1-SO₄), an inert form of estrogen that will not enter target cells.
- 2) 2-hydroxy catechol estrogens are safer than 4-hydroxy catechol estrogens; these are created from the cytochrome enzyme CYP1A1, which is activated by phytochemicals found in cruciferous vegetables (cauliflower, broccoli, cabbage), and by iodine, progesterone, and Vitamin D. If 2-hydroxy catechol estrogens are low, or are low relative to the 4-hydroxy catechol estrogens, consider lowering consumption of meats and increasing consumption of green leafy and cruciferous vegetables.

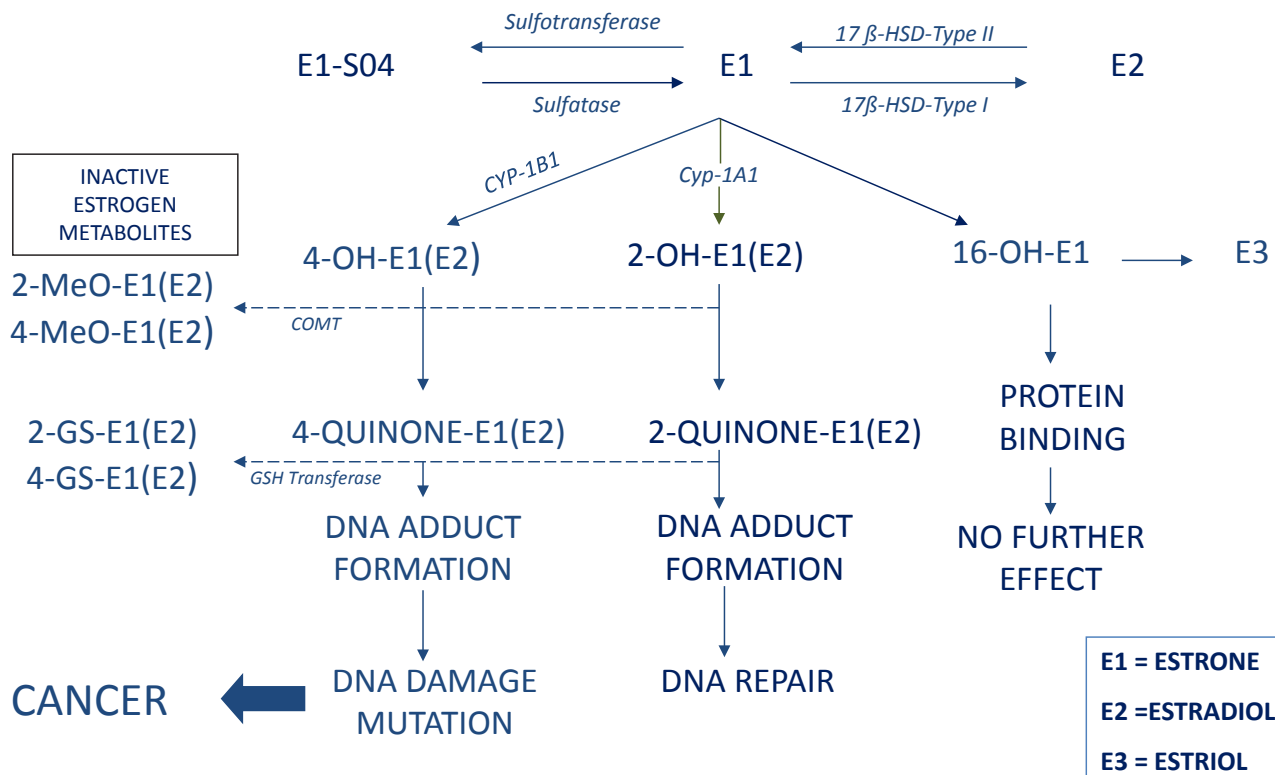
- 3) 4-hydroxy catechol estrogens are created from the cytochrome enzyme CYP1B1, which is induced by man-made petrochemical toxins (some drugs, oils, plastics, pesticides, household chemicals, etc.) that contaminate our food, water, and air. If 4-hydroxy catechol estrogens are elevated, consider identifying and avoiding exposure to these petrochemical products as much as possible.
- 4) As the good (2-OH-catechols) and bad (4-OH-catechols) are both rendered inactive by COMT-mediated methylation, it is important to maintain adequate substrates for COMT. These include vitamins B6, B12, and folate, as well as betaine. Excessive estrogens tend to deplete these vitamins, so supplementation during times of estrogen excess (often at perimenopause) is vital to clearing catechol estrogens such that they are less likely to spill over into the highly reactive and mutagenic 4-estrogen quinones.
- 5) Prevent the conversion of the 2- and 4-catechol estrogens into their respective quinones, and provide adequate substrate to inactivate them if they do. The 2- and 4-catechol estrogens are activated to their quinones by oxidized fats and some heavy metals such as arsenic and mercury. Removal of bad (trans) fats from the diet and countering the heavy metals with adequate beneficial elements such as iodine and selenium are important for preventing further oxidation of estrogen catechols to estrogen quinones.
- 6) Iodine, progesterone (only when estrogens are within normal to high physiological range of a premenopausal woman), and Vitamin D have all been shown to increase formation of 2-hydroxy catechol estrogens, and decrease the relative concentration of

the more dangerous 4-hydroxy catechol estrogens. Consider supplementation if any of these is found to be low by testing.

- 7) Glutathione is important as the last step in detoxification of quinone estrogens. Excessive medications, hormone therapy, and exposure to environmental toxins such as heavy metals, cigarette smoke and excessive industrial air pollution results in high utilization and lower levels of glutathione. Cysteine is the most limiting amino acid as regards glutathione synthesis, and vitamin C is an essential nutrient for reactivation of oxidized glutathione. Selenium also plays an important role in glutathione's effectiveness as an anti-oxidant, and low levels of selenium have been associated with higher risks for cancers of the breasts and prostate. If quinone estrogens are elevated, particularly if the methylated catechol estrogens are low, consider foods high in sulfur containing amino acids (allium foods like garlic and onions) and/or supplementation with N-acetylcysteine and Vitamin C. Also consider supplementation with selenium, an essential element in many anti-oxidant enzymes, if it is found to be low.
- 8) Inadequate production of melatonin has been linked with breast cancer. Melatonin has antiestrogenic actions, acting as a selective estrogen receptor modulator in breast tumor cells, and also down-regulating aromatase, thus reducing local estrogen synthesis from androgenic precursors (9). Because of the oncostatic effects of melatonin, breast cancer risk can be reduced by getting adequate sleep and/or reducing exposure to light at night.



ESTROGEN METABOLISM AND BREAST CANCER RISK



Shown in this figure are the stages of estrogen (E1 and E2) metabolism that lead to harmless (2-OH-E1, 2-OH-E2, 16-OH-E1, and E3) or potentially toxic (4-OH-E1 and 4-OH-E2) estrogen metabolites. Both 2- and 4-OH estrogens (often referred to as catechol estrogens) are inactivated by methylation with COMT (Catechol-Ortho-Methyl-Transferase) to form 2- and 4-methoxy estrogens (2-MeO-E1 and -E2 or 4-MeO-E1 and -E2). If methylation does not occur due to lack of COMT substrates (vitamins B6, B12, folate, betaine), or defective gene polymorphisms in COMT, the catechol estrogens can further oxidize to 2- and 4-estrogen quinones. Formation of these oxidized quinone estrogens occurs more readily in the presence of excessive oxidized lipids, such as trans-hydrogenated fats, and heavy metals. Under ideal situations the quinone estrogens react with glutathione, which inactivates them. In the absence of adequate cellular glutathione and glutathione transferase, these highly reactive and electrophilic estrogen quinones bind covalently to DNA forming adducts that can lead to mutations that increase risk for cancers in estrogen target tissues such as the breasts, uterus, ovaries, and prostate.

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