Estronex™

Estronex℠ Profile Guide

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Analytes:

2-Hydroxyestrone (2-OHE1)
2-Hydroxyestradiol (2-OHE2)
4-Hydroxyestrone (4-OHE1)
16α-Hydroxyestrone (16α-OHE1)
2-Methoxyestrone (2-OMeE1)
4-Methoxyestrone (4-OMeE1)
Ratios
(2-OHE1+2-OHE2)/16α-OHE1
2-OHE1/2-OMeE1

Testing

The Estronex Profile is an easy-to-use and cost-effective way to assess estrogen metabolism. While estrogen-sensitive cancers are caused by multiple genetic and environmental factors, how effectively a woman metabolizes and eliminates estrogens gives information about her risk of developing certain cancers. Breast cancer is the second most common cause of cancer death in women.[1] As part of a breast cancer prevention program, women of all ages may benefit from the Estronex Profile. Women age 40 and older may consider taking the test regularly in addition to routine breast cancer screening methods. Breast cancer survivors may benefit from regular assessment of the Estronex Profile to prevent recurrence. The Estronex Profile can also be used to evaluate men at risk of developing breast and prostate cancer.[2,3]

Imbalances In Estrogen Have Been Found In The Following Conditions:[4-15]

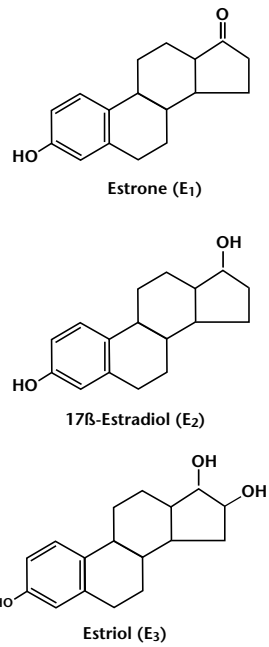
- Breast cancer
- Colorectal cancer
- Prostate cancer
- Uterine cancers, including endometrial
- Ovarian cancer
- Cervical cancer
- Head and neck cancers
- Polycystic ovarian syndrome
- Uterine fibroids
- Endometriosis
- Preeclampsia
- Osteoporosis
- Menopausal symptoms
- Premenstrual syndrome

The Estronex Profile requires only a first morning urine collection. Research has shown that the 2/16 ratio is stable over the menstrual cycle but it is recommended that the test be completed at the same time in the menstrual cycle when comparing before- and after-treatment tests. After initiation of treatment, retesting is recommended within three to six months until the ratio is within normal limits, and then annually or biannually thereafter depending on degree of risk. Chemotherapeutics targeting estrogen or estrogen receptors may also change estrogen metabolism and monitoring the effects of such agents on estrogen levels may be useful.

Background

Estrogen's basic biological function is as an anabolic hormone. There are three primary forms of estrogen:[19] estrone (E1); estradiol (E2); and estriol (E3). All three forms have varying levels of activity and specificity for different tissues. E2 is the most active of the estrogens and is responsible for most of the actions attributed to the estrogens.

Because hormones exert great biological activity in small doses, it is very important that the body effectively eliminates them. Estrogens are cleared by phase I and phase II detoxification in the liver. In phase I, the estrogens are oxidized by the cytochrome P450 (CYP450) enzyme

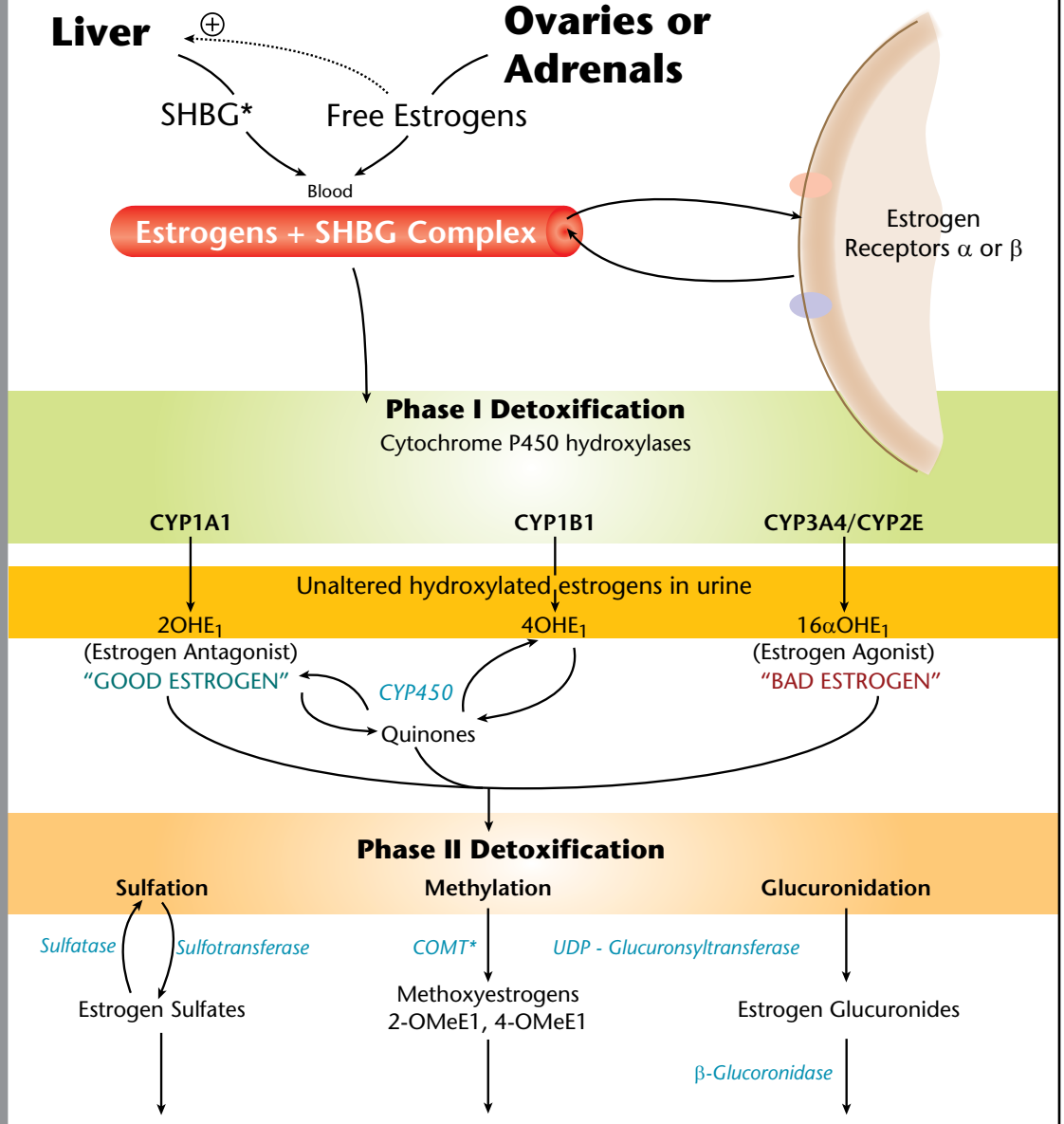


Estrogen Pool:
Free Estrogens
& SHBG-bound
Sulfated Estrogens
Estrogen Metabolites
Exogenous Estrogen
Reabsorbed Estrogens

Estrogen Actions:
Estrogen can have a stimulant or agonist effect depending on the receptor and/or the tissue. Activated estrogen receptors modify expressions of gene cassettes.

Estrogen Clearance:
Phase I Detoxification products may be modified by Phase II enzymes.

Phase II Detoxification products are water soluble, non-reacting, can be excreted in the urine or bile, and do not have the hormonal effects of their parent molecules.



EXCRETION

Enterohepatic Recirculation
Intestinal bacterial ß-glucuronidase cleaves glucurorides, freeing active estrogens to be reabsorbed, increasing total estrogen load.
Enhancing good bacteria or supplementing with D-glucarate can reduce intestinal glucuronidase effects. Adding dietary fiber can increase excretion of estrogen gluronidase.

Cancer Risk Factors
Low 2/16 ratios or high 4-OHE1 may increase risk of estrogen-sensitive cancers. See comments on dietary factors and nutritional supplements that can help to normalize these abnormalities.

*SHBG = sex hormone-binding globulin
*COMT = catechol-O-methyltransferase

FIGURE 1. ESTROGEN METABOLISM

system (Figure 1). When CYP450 enzymes act on estradiol, the products are: 2-hydroxyestrone (2-OHE1), 4-hydroxyestrone (4-OHE1), and 16α-hydroxyestrone (16α-OHE1).[16] Metabolism by CYP1A1 produces 2-OHE1, CYP1B1 produces 4-OHE1, and CYP2E family produces 16α-OHE1.[18] There are large variations in how individual women metabolize estrogen, which may be related to single nucleotide polymorphisms (SNPs) in these enzymes.

In women not on hormone-replacement therapy, 2-OHE1 is considered a "good" estrogen because it is associated with reduced cancer growth.[17] 16α-OHE1 has been shown to encourage tumor development and 4-OHE1 might cause DNA damage. Both 16α-OHE1 and 4-OHE1 have been referred to as the "bad" estrogens.[18-19] Poor ability to form 2-OHE1 leads to greater production of 16α-OHE1.

In post-menopausal women on hormone-replacement therapy, a very high 2-OHE1 can result from high doses of estradiol. Thus, elevated levels of 2-OHE1 are associated with increased cancer risk.[22] The prescribing doctor should be consulted about dosing when such very high 2-OHE1 are found.

INTERPRETATIONS

RATIOS

HYDROXYESTROGENS RATIO

Studies have shown that measuring the ratio of 2-hydroxylated estrogens (2-OHE1 + 2-OHE2) to 16α-OHE1 (2/16 ratio) provides an important indication of risk for future development of breast cancer.[20] This ratio of "good to bad estrogen" can be determined from a single urine specimen. Studies have shown that individuals with breast cancer have lower 2/16 ratios. A low 2/16 ratio may indicate increased long-term risk for other estrogen-sensitive cancers including uterine, ovarian, cervical, prostate, and even head and neck cancers.[18,21] Though a woman's biochemical individuality certainly affects how much of each form of estrogen is produced, in many women the primary cause of a low 2/16 ratio is related to dietary factors. Imbalances in the 2/16 ratio have been associated with prostate cancer,[23] colorectal cancer, and polycystic ovarian syndrome.[24]

Low 2/16 Ratio

Urinary 2-OHE1 should be high enough relative to the urinary levels of 16α-OHE1, to yield a 2/16 ratio that is greater than the low limit shown on the chart in the report.

Treatments To Increase 2/16 Ratio

- Decrease coffee consumption and smoking
- Lower an elevated BMI
- Supplement with indole-3-carbinol (I3C) or di-indolemethane (DIM)
- Increase cruciferous vegetables including: broccoli, cabbage, and Brussels sprouts
- Supplement with dried organic Brussels sprouts and kale
- Increase ground flax seed
- Increase intake of fish oils (omega-3 fatty acids)
- Increase fruit and vegetable intake
- Soy isoflavones (considered mildly estrogenic and may not be ideally suited for patients with hormone-sensitive cancers)
- Consider alternatives to oral contraceptive use (oral contraceptive users had significantly lower 2/16 ratios)

- Decrease inflammation and oxidative damage:
  - » Evaluate markers for oxidative stress, such as p-hydroxyphenyllactate, 8-hydroxy-2'-deoxyguanosine, and lipid peroxides
  - » Increase antioxidants in foods or supplements
- Ensure adequate estrogen detoxification and biotransformation:
  - » Sulfur-rich compounds such as N-acetyl cysteine, calcium D-glucarate and methyl donors such as S-adenosylmethionine (SAMe), vitamin B_{12}, folic acid, and vitamin B_6
  - » Ensure effective GI elimination:
    - › Use 4R Protocol*
    - › Increase fiber, fruits, and vegetables
    - › Consume a low-allergen diet

**** Refer to Metametrix Handbook page 133***

High 2/16 Ratio

Most, but not all, epidemiological studies have found that women with a high 2/16 ratio are at reduced risk for breast cancer, however, excessive reduction of 16α-OHE1 may reduce bone formation and increase oxidant stress.

Treatments to Decrease 2/16 Ratio

- Consider decreasing therapies that increase the 2/16 ratio (see above)
- Increase antioxidants
- Check methylation activity (homocysteine, methylmalonate, formiminoglutamate), accumulation of 2-OHE1 may demonstrate poor methylation activity
- Check bone mineral density and follow with the deoxypyridinoline (DPD) test

METHYLATION RATIO

In phase II detoxification, the estrogen metabolites are conjugated with compounds that allow excretion by the body. Adequate methylation is very important to properly clear estrogens. High catechol-O-methyltransferase (COMT) activity has been associated with reduced risk for certain cancers. 2-Hydroxyestrone and 4-hydroxyestrone are methylated in Phase II to produce 2-methoxyestrone (2-OMeE1) and 4-methoxyestrone (4-OMeE1). 2-OMeE1 has anticancer effects and studies show that it is lower in breast cancer patients than controls. It has been referred to as a "good" estrogen. 4-OMeE1 is a non-carcinogenic metabolite of 4-OHE1.

High 2-OHE1/2-OMeE1 Ratio

- Imbalanced estrogen metabolism
- Low activity COMT
- Evaluate methylation activity:
  - » COMT genetic testing
  - » Homocysteine
  - » B_{12}
  - » Folic Acid
  - » SAMe
  - » B_6
  - » Reduce stress: COMT is involved in the metabolism of epinephrine, reducing availability for estrogen metabolism

ANALYTES

While the ratios are important, the clinician should note the values of the individual estrogens. Ideally, the patient's levels of individual estrogen metabolites will be within normal limits for the population group (premenopausal, post-menopausal without hormone therapy, post-menopausal with hormone therapy, or males).

2-HYDROXYESTRONE (2-OHE1)

High 2-OHE1

- High levels of the "good estrogen" are typically considered beneficial; however, when coupled with a low 2-OMeE1, it may indicate poor methylation activity (see high 2-OHE1/2-OMeE1).
- If both 2-OHE1 and 16α-OHE1 are high, then total estrogen load may be high. Consider methods to better clear the estrogens from the body or HRT dosing may need revision if only 2-OHE1 is very high.

Low 2-OHE1

- Low levels of the "good estrogen" (see treatments to increase 2/16 ratio on page 3)
- If 2-OHE1 is low and 16α-OHE1 is low, then total estrogen production may be low. Address underlying causes of low hormone production, as needed.

16 ALPHA–HYDROXYESTRONE (16α-OHE1)

High 16α-OHE1

- High levels of the "bad estrogen" (see treatments to increase 2/16 ratio)
- If both 2-OHE1 and 16α-OHE1 are high, then total estrogen load may be high. Consider methods to better biotransform and excrete the estrogens from the body or decrease HRT.

Low 16α-OHE1

- Low levels of the "bad estrogen" are typically considered beneficial.
- If 2-OHE1 is low and 16α-OHE1 is low, then total estrogen production may be low. Address underlying causes of low hormone production.

2-METHOXYESTRONE (2-OMEE1)

2-OMeE1 is produced from 2-OHE1 through the COMT enzyme. Anti-cancerogenic effects have been ascribed to 2-OHE1and particularly 2-OMeE1.[23] 2-OMeE1 has shown antiproliferative effects in both hormone-dependent and hormone-independent breast cancer cells.[25,26] These studies have shown that urinary 2-OMeE1 levels were lower in breast cancer patients than controls.

High 2-OMeE1

- Generally no treatment recommended
- Ensure adequate phase II detoxification

Low 2-OMeE1

Identify cause:

- If 2-OHE1 and 4-OHE1 are high, then low 2-OMeE1 is likely the result of poor methylation. Follow methylation treatment recommendations.
- If 2/16 ratio is also low, then low 2-OMeE1 may be indicating a CYP imbalance. Follow recommendations for improving a low 2/16 ratio.
- If total estrogen metabolite production is low, then consider direct assessment of estrogen levels.
- Ensure adequate COMT function via methyl donors such as SAMe, B_{12}, folic acid, and B_6.
- Poor cancer protection
- Associated with preeclampsia

4-HYDROXYESTRONE (4-OHE1)

4-OHE1 is referred to as the "bad" estrogen, along with 16α-OHE1. They are primarily produced by CYP1B1 and CYP34A, respectively, enzymes localized in tissues, including breast and prostate as well as liver. Some have suggested that increased expression of CYP1B1 and 4-hydroxylation of estradiol are biomarkers of tumorigenesis.[27,28] 4-OHE1 may be further metabolized to DNA-damaging quinone estrogens.[29] It may also have estrogenic effects.[30] Human breast cancer tissue produces much higher levels of 4-OHE1 than 2-OHE1, while normal breast tissue produces approximately equal amounts of the two metabolites. Women taking hormone therapy with a polymorphism in CYP1B1 had twice the risk of developing breast cancer compared to other HRT users.[31]

High 4-OHE1

- High levels of the "bad estrogen"
- Improve methylation by adding cofactors (B_{12}, folate) or methyl donors (betaine, dimethyl glycine)
- Consider genetic testing for COMT and CYP1B1 activity, particularly if positive family history
- Reduce stress: COMT is involved in the metabolism of epinephrine, reducing availability for estrogen metabolism
- Increase inhibitors of CYP1B1
  - » Grapefruit
  - » Ginseng
- See treatment for 2/16 ratio
- Avoidance of CYP1B1 inducers
  - » Polycyclic aromatic hydrocarbons
- Evaluate methylation activity:
  - » Serum Homocysteine
  - » Serum B_{12} or methylation
  - » Urinary FIGLU
  - » Urinary xanthurenate
- Other potential protective factors:
  - » Glutathione (reduction of estrogen quinones)
  - » Resveratrol (prevents estrogen quinone formation)
  - » Selenium, zinc, magnesium

Low 4-OHE1

- Low levels of the "bad estrogen"
- Generally no treatment recommended

4-Methoxyestrone (4-OMeE1)

4-OMeE1 is the COMT-detoxified, non-carcinogenic product of 4-OHE1.[29] Low levels of 4-OMeE1 were found in the urine of both high-risk and breast cancer patients.[32] It may be protective to have higher levels of the deactivated product, 4-OMeE1.

High 4-OMeE1

- Generally no treatment recommended

Low 4-OMeE1

- Improve methylation, see treatments for high 4-OHE1

Other Testing

There are other laboratory tests that reveal important information about a patient's estrogen metabolism. Higher levels of inflammation can impact estrogen receptor reactivity.[33] Oxidative stress should be assessed to assure adequate hormone function. Markers for oxidative stress are p-hydroxyphenyllactate, 8-hydroxy-2'-deoxyguanosine, high sensitivity C-reactive protein (hs-CRP), and lipid peroxides. Tests to evaluate levels of anti-inflammatory fatty acids can also be useful. Hormones excreted in bile can be deconjugated by intestinal bacteria and reabsorbed in cases of gastrointestinal dysbiosis. If dysbiosis is suspected, consider a GI Effects℠ Profile, Organix℠ Dysbiosis Profile, and an Allergix℠ Food Antibody Profile. Evaluating xenoestrogen levels (Phthalates & Parabens Profile) or single nucleotide polymorphisms (SNPs) in detoxification enzymes could be helpful when treatments to improve estrogen clearance are ineffective. Finally, a full urinary hormone profile may be indicated to evaluate a patient's levels of other hormones.

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