Estrogen Metabolism 101

A Practical Guide to Understanding and Measuring

Dr. Hilary Miller, ND

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Outline

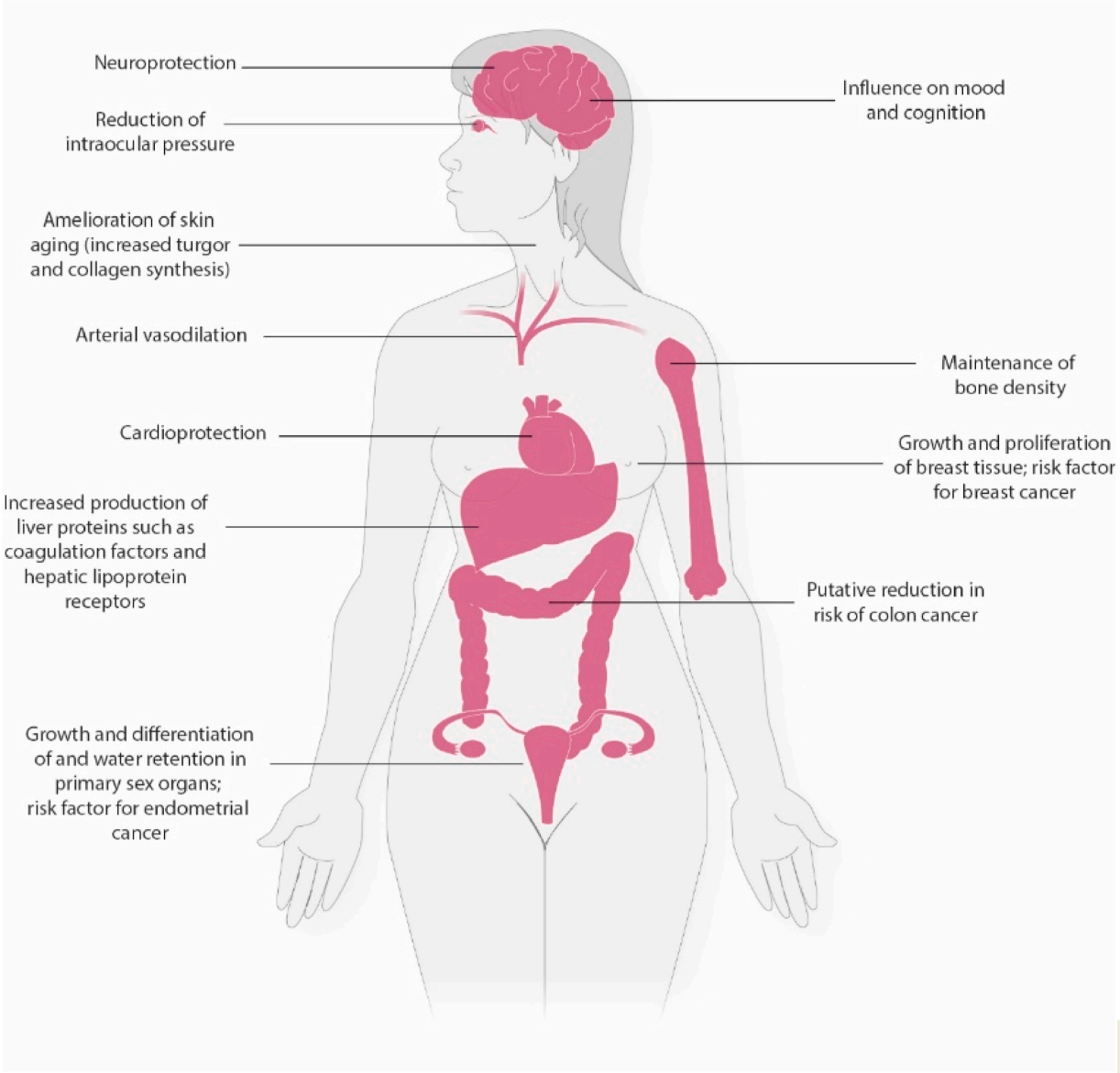
1. **Overview:** What is Estrogen Metabolism?
2. **Estrogen Metabolites:** What they do, and a high-level overview of the literature.
3. **Interpretation:** How to determine when metabolite levels are abnormal.
4. **Research:** Discover what the research says about these ratios.
5. **Examples:** Review cases for examples of common patterns.

What Does Estrogen do?

- Estrogen is a female reproductive hormone that is important at all stages of life.
- It has significant impacts on many body systems.
  - Promotes the growth and development of the endometrial tissue.
  - Promotes breast development and tissue differentiation.
  - Increases muscle mass, bone density
  - It is anti-inflammatory and anti-oxidant
  - Supports healthy weight, metabolism, and insulin function
  - We also see significant benefits for skin, hair, and mood

  - Involved in the development of breast and endometrial cancer

What Does Estrogen do?



Biason-Lauber A, Lang-Muritano M. 2022;554:111710.

What Does Estrogen do?

- Estrogen negatively impacts the course of some diseases:
  - Breast cancer (BC)
  - Breast tumors
  - Endometriosis
  - Endometrial hyperplasia
  - Endometrial cancer
  - Uterine fibroids
  - Etc.

Breast Cancer

- Breast Cancer (BC) accounts for 12.5% of all cancer diagnoses (this includes in men, for whom breast cancer is extremely rare).
- In the US, BC accounts for 30% of diagnosed female cancers.
- Advancements in detection, prevention, and treatment have made BC less deadly than it used to be (HOORAY!!) but it is still a serious health concern among women.

Estrogen and Breast Cancer

- About 1 in 8 average-risk women will face a BC diagnosis
- Non-modifiable BC risk factors:
  - Age: Median age at diagnosis is 62
  - Female sex (estrogen is implicated)
    - Starting puberty earlier and going through menopause later increase lifetime estrogen exposure and increase BC risk
  - Family history (15% of diagnoses have a family history)
    - If you have a family history your risk doubles
  - Genetics account for 5-10% of BC diagnoses
    - BRCA1 and BRCA2 are the most common.
    - If you have one of these gene mutations, you have around 70% chance of getting breast cancer.
    - 90% of breast cancers do not have BRCA1 or 2 gene mutations.
  - Ethnicity impacts BC risk

https://www.breastcancer.org/facts-statistics

Estrogen and Breast Cancer

- Modifiable BC risk factors:
  - Increases risk:
    - **Smoking**
    - **Alcohol consumption**
    - **BMI**
    - **Certain types of HRT (progestins, MPA)**
    - **Exposure to certain environmental chemicals**
    - Low vitamin D
  - Decreases risk:
    - **Exercise**
    - **Maintaining a healthy weight**
    - **Eating a Mediterranean diet high in fiber and plant foods**
    - **Avoiding alcohol and tobacco products**
    - Breastfeeding for more than one year before the age of 35

Highlighted in GREEN = impact estrogen metabolism

https://www.breastcancer.org/risk/risk-factors

Estrogen and High Estrogen Conditions In Females

- Modifiable risk factors in endometrial cancer, uterine fibroids, and heavy menses:
  - Increases risk:
    - **Smoking**
    - **Alcohol consumption**
    - **Being overweight or obese**
    - **Exposure to certain environmental chemicals**
    - Low vitamin D (Increased BC and fibroid risk)
    - **Excess estrogen exposure**
  - Decreases risk:
    - Adequate progesterone
    - **Exercise**
    - **Maintaining a healthy weight**
    - **Eating a Mediterranean diet high in fiber and plant foods**
    - **Avoiding alcohol and quitting tobacco use as early as possible**

Highlighted in GREEN = impact estrogen metabolism

https://www.cancer.org/cancer/types/endometrial-cancer/causes-risks-prevention/risk-factors.html
https://www.breastcancer.org/risk/risk-factors

Assessing Estrogen

- How do we assess estrogen levels?
  - Test parent estrogens (E1 and E2)
    - Know about timing of estrogen cycles and their appropriate reference ranges.

    - Serum
    - DUTCH urine testing
      - Cycle mapping for estrogen levels over a cycle
      - DUTCH Complete or DUTCH Plus for a one-day test

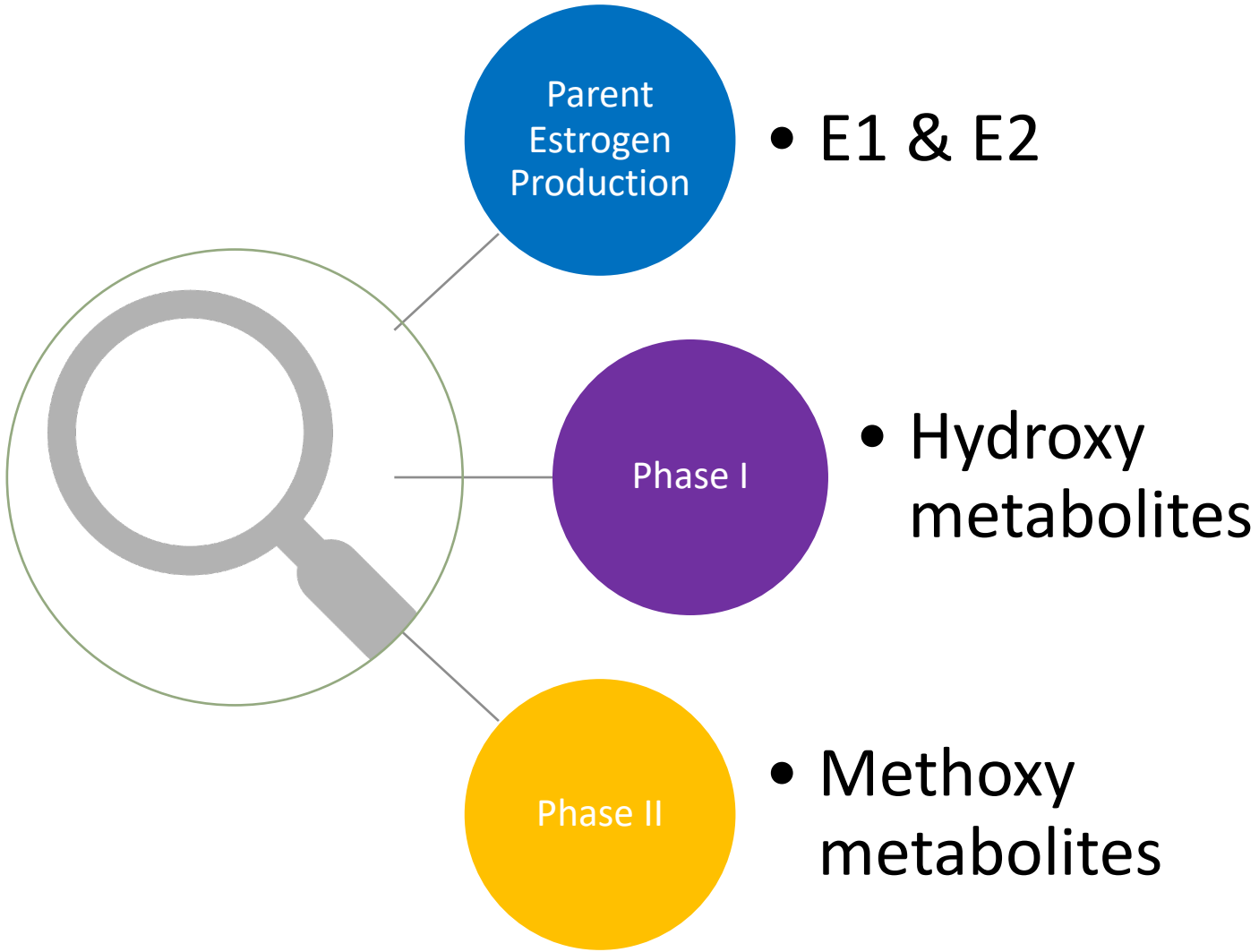
Assessing Estrogen

Removal, or detoxification is one mechanism that controls the impact of estrogen on the body.

- Estrogen is detoxified through phase I, II, & III detox pathways.
- The genetics, epigenetics, and efficiency of these pathways affect estrogen levels.

Estrogen production → **Estrogen excretion**

Estrogen Metabolism:



- E1 & E2
- Hydroxy metabolites
- Methoxy metabolites

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Estrogen Metabolism:

Estrogen moves passively into cells:

- Acts on the cell membrane and within the cell
- Requires transformation to move it out of the body: detoxification (detox)

Phase I: The first step of detox is hydroxylation (OH) through CYP450 enzymes.

- The main estrogen metabolizing enzymes are:
  - CYP1A1, CYP1A2 → 2-OH-E1 and 2-OH-E2 (catechol)
  - CYP3A4 → 16-OH-E1 and 16-OH-E2 (or Estriol, E3)
  - CYP1B1 → 4-OH-E1 and 4-OH-E2 (catechol)

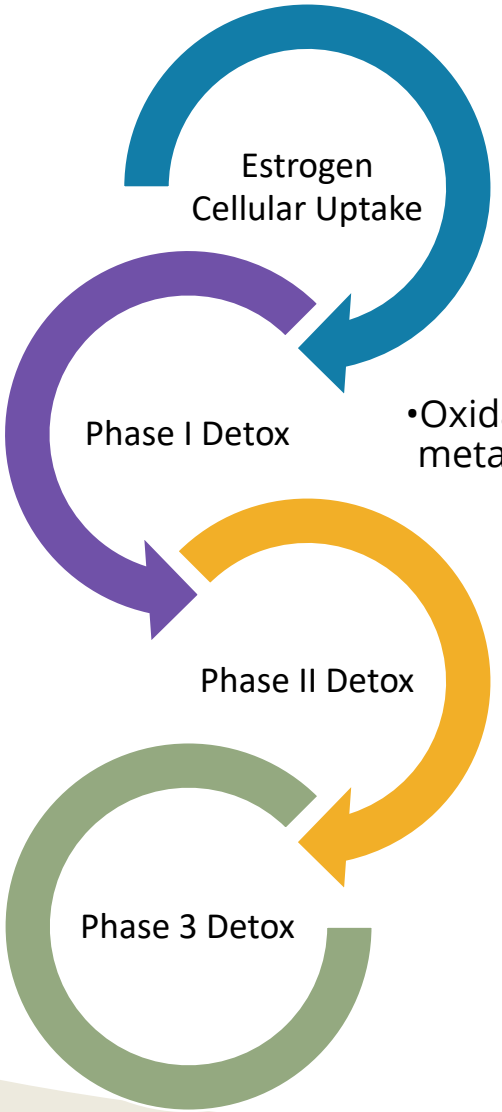
Phase II: The second step methylation and conjugation

- Catechol-O-Methyltransferase (COMT)
  - Methylation → Catechol-O-Methyltransferase (COMT)→ 2Me-E1, 2Me-E2, 4Me-E1, 4Me-E2
- Conjugation (glucuronidation, sulfation)
  - This uses a variety of enzymes and can happen at any stage. For example, a major component of urinary E1 is E1-S (estrone sulfate).

Phase III: The third step is elimination

- Serum → Kidneys→ **Urine**
- Bile→ Intestines →Feces/Stool

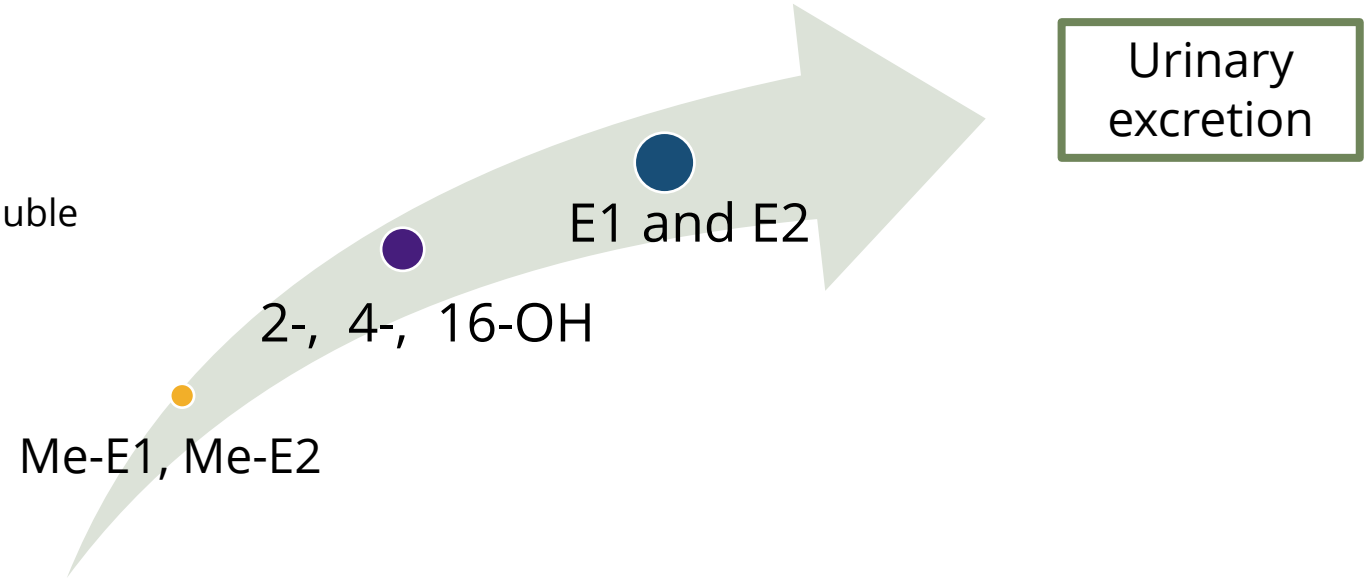
Estrogen Metabolism:



- Liver
- Target Tissues (Breast, endometrium, bone, skin, muscle, pancreas, liver, cardiovascular tissue, etc…)

- Oxidative metabolites

Neutral, water-soluble metabolites



Phase 1

What Is Phase I Estrogen Metabolism?

"Cytochrome **P450 enzymes** that metabolize estrogens are expressed in the mammary gland, uterus, brain, and other **target tissues** for estrogen action, and this results in the formation of h**ydroxylated estrogens** in these tissues. Estradiol metabolites formed in the target tissues ***at or near* estrogen receptors** may either be inactive or have **important biological effects**, and changes in the activities of estrogen-metabolizing enzymes in target tissues may **profoundly influence estrogen action**."

Zhu BT and Conney AH Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis. 1998; 19: 1-27.

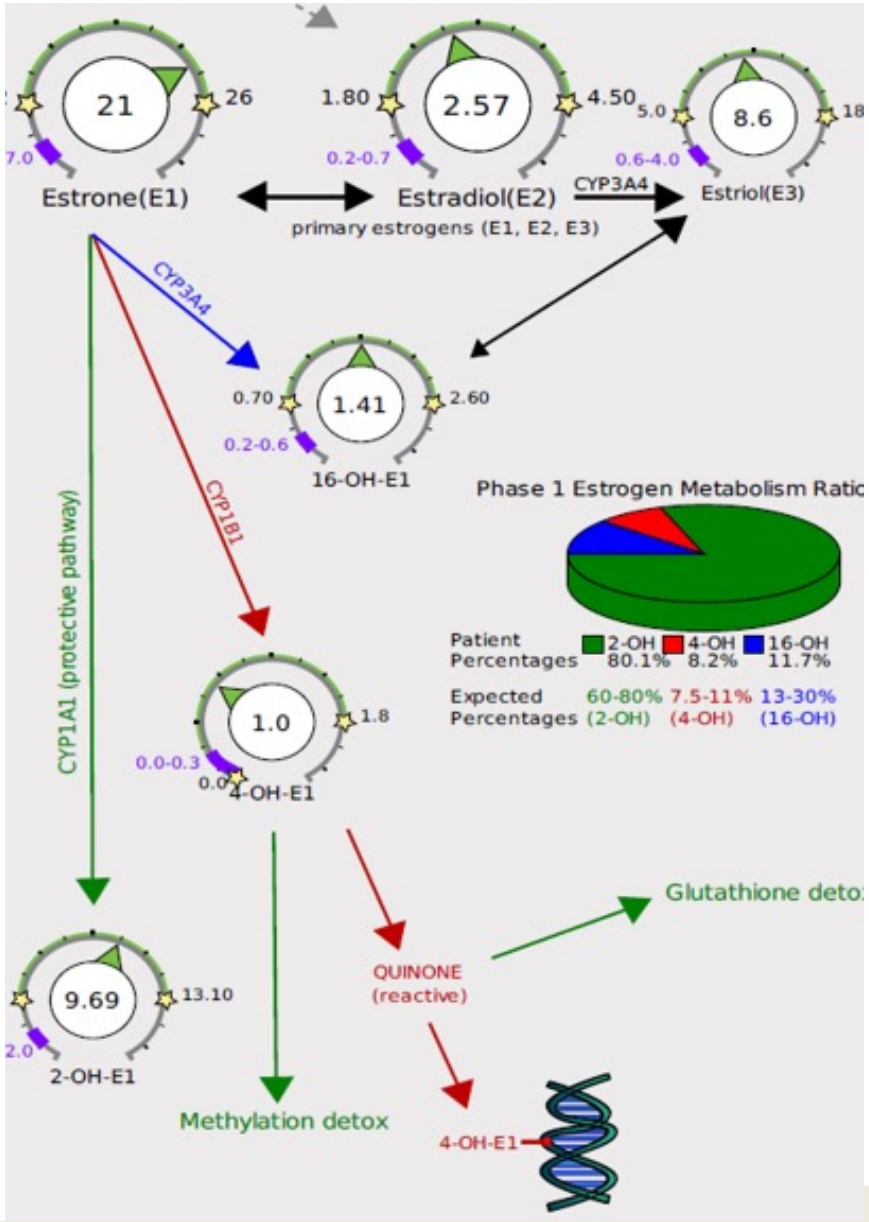
What is Phase I Estrogen metabolism?

Parent estrogens in phase I are broken down via 3 main pathways.

The **green arrow** coming out of estrone shows the **2OH pathway,** the happy pathway.

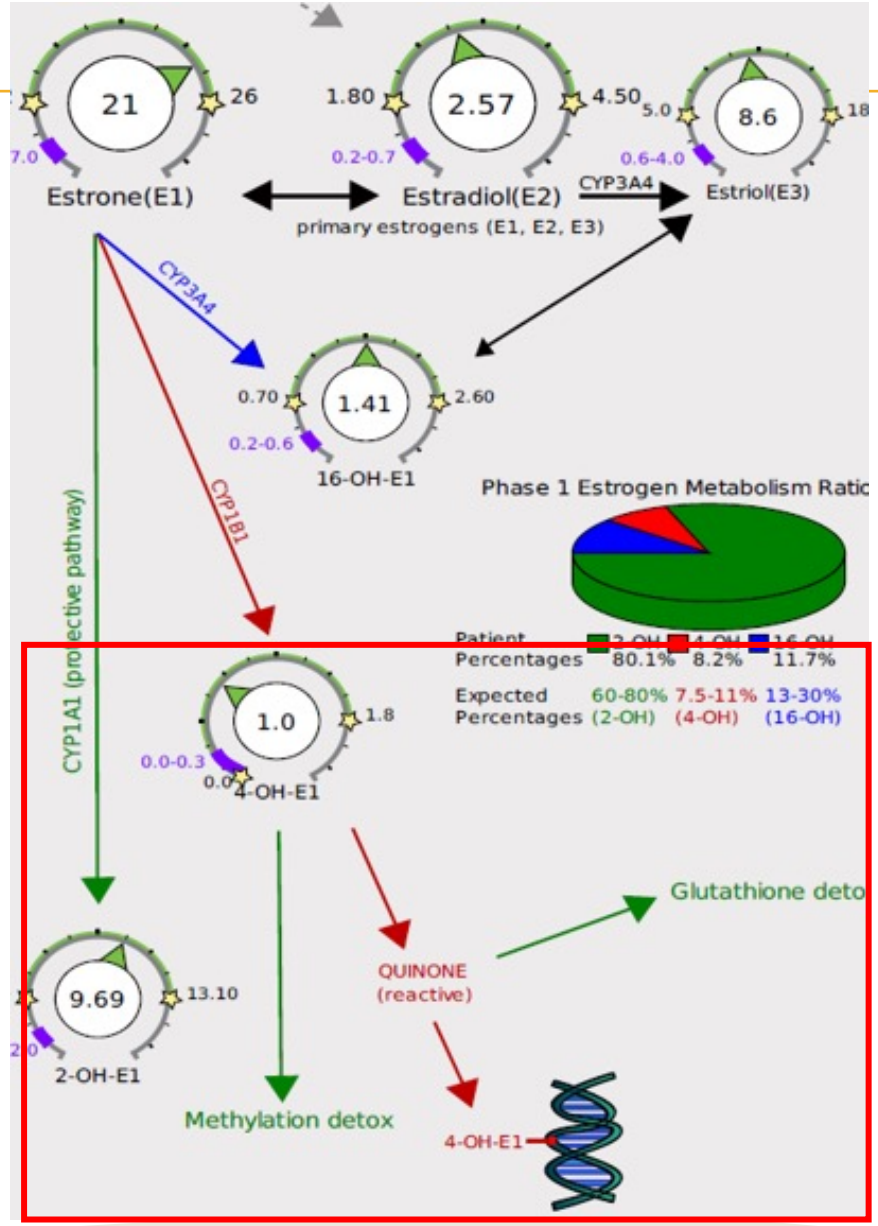
The **red arrow** coming out of estrone shows **4OH** which is the bad pathway: Quinone formation, **oxidative DNA damage.**

The **blue arrow** coming out of estrone points to **16OH,** which is **estrogenic.**



Phase I Estrogen Metabolism

- The **16OH metabolites are sulfated** for removal.
- 2- and 4- OH metabolites are also known as “Catechol estrogens”.
- They can be oxidized:
  - Oxidative enzymes (any)
  - Metal ions
- Oxidation creates quinones and semi-quinones, which create reactive oxygen species (ROS)
- ROS can cause oxidative damage to DNA and lipids.
- Once **methylated** (COMT enzyme), **catechol estrogens are neutral** and have no further oxidative potential.
- In the presence of antioxidants, the oxidative potential is minimized.
  - NAC, glutathione
  - Resveratrol
  - Green tea extract



Chang M. BMB Rep. 2011;44(7):423-434.

Phase I Estrogen Metabolism

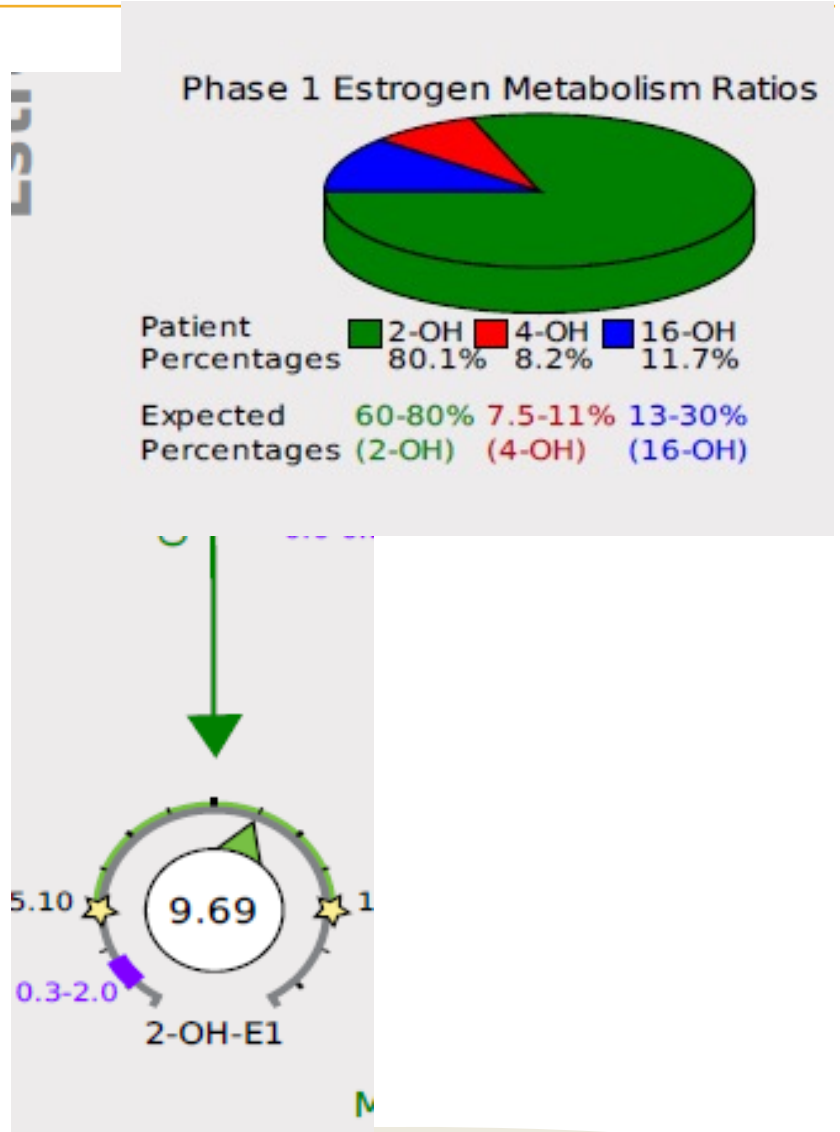
- Phase I occurs in both in the liver and in estrogen-metabolizing cells, leaving estrogen-sensitive tissues vulnerable to the damaging effects of ROS:
  - Liver
  - Uterus
  - Breast
  - Cardiovascular tissue
  - Lungs
  - Brain, etc.

Chang M. BMB Rep. 2011;44(7):423-434.

2OH Pathway

2OH pathway

- **2OH** estrogens are formed from **CYP1A2** in the liver and 1A1 in extrahepatic tissues:
  - Breast
  - Uterus
  - Placenta
  - Brain
- 2OH metabolites are **anti-carcinogenic**
- 2OH metabolites have an **inhibitory effect** on cell proliferation
- 2OH metabolites are **methylated at a faster rate** than 4OH (stabilized quickly)
- Stable→ less oxidative.
- Readily moved into Phase II.
- This pathway is associated with lower breast cancer risk.



Tsuchiya Y, et al. Cancer Lett. 2005;227(2):115-124.

2OH Pathway

6,915 samples from the **Columbia Missouri Serum Bank**, women were followed for incident breast cancer from 1977-2002.

From those samples, 215 BC cases and 215 matched controls were compared for estrogen metabolite patterns.

Patients were postmenopausal women who had not used HRT.

Greater amounts of 2OH estrogens were associated with reduced BC risk.

RESEARCH ARTICLE Open Access

Relationship of serum estrogens and estrogen metabolites to postmenopausal breast cancer risk: a nested case-control study

Roni T Falk[1*], Louise A Brinton[1], Joanne F Dorgan[2], Barbara J Fuhrman[3], Timothy D Veenstra[4], Xia Xu[4] and Gretchen L Gierach[1]

Falk RT, et al. Breast Cancer Res. 2013;15(2):R34.

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2OH pathway

- Early methods of measuring estrogen metabolism used direct immunoassays, which is not accurate enough especially for postmenopausal women with very low levels.
- Novel, but more accurate LC-MS/MS method was used which can detect much lower levels of metabolites.
- Serum samples were used for this study.

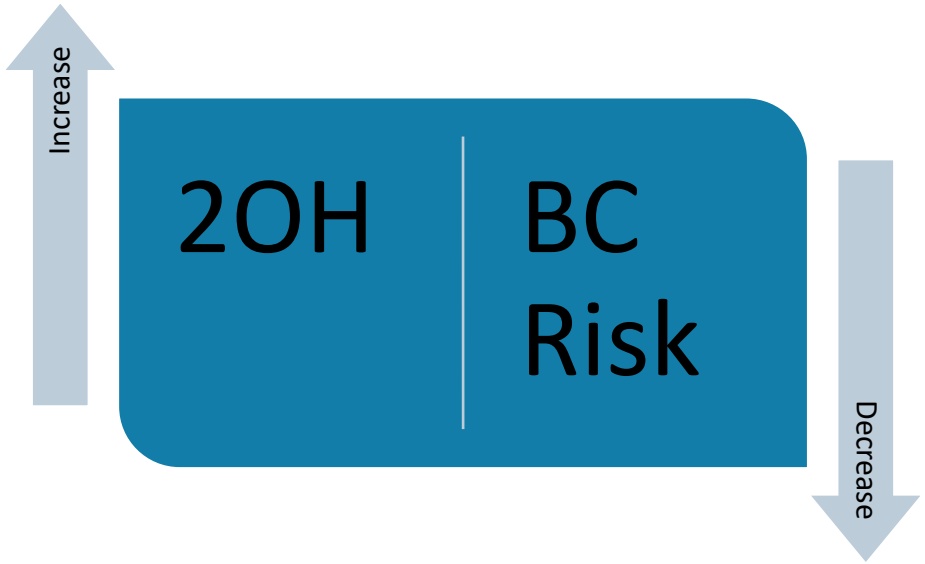
Higher 2OH/parent estrogens were associated with a 28% reduction in BC risk.

Published in final edited form as:
Steroids. 2015 July ; 99(Pt A): 67–75. doi:10.1016/j.steroids.2015.02.015.

Epidemiologic Studies of Estrogen Metabolism and Breast Cancer

Regina G. Ziegler[1], **Barbara J. Fuhrman**[1,2], **Steven C. Moore**[1], and **Charles E. Matthews**[1]

[1]Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892-9773, USA



Ziegler RG, et al. 2015;99:67-75.

2OH pathway

Moore et al. Shanghai Women's Health Study Cohort

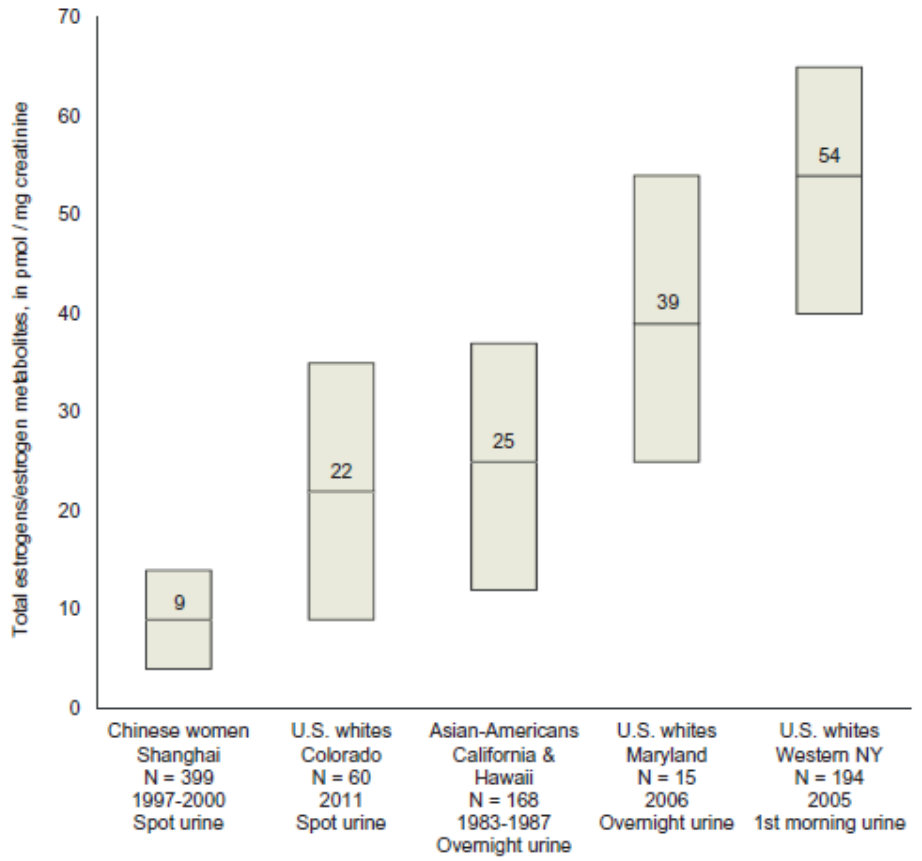
399 postmenopausal invasive breast cancer patients

399 matched controls

Urinary concentrations of E2, E1, and 13 estrogen metabolites were measured using **LC-MS/MS instruments.**

Lower urinary parent estrogen concentrations were found in Chinese women compared to American women (whether white or Asian American).

Higher 2OH metabolites were associated with decreased BC risk.

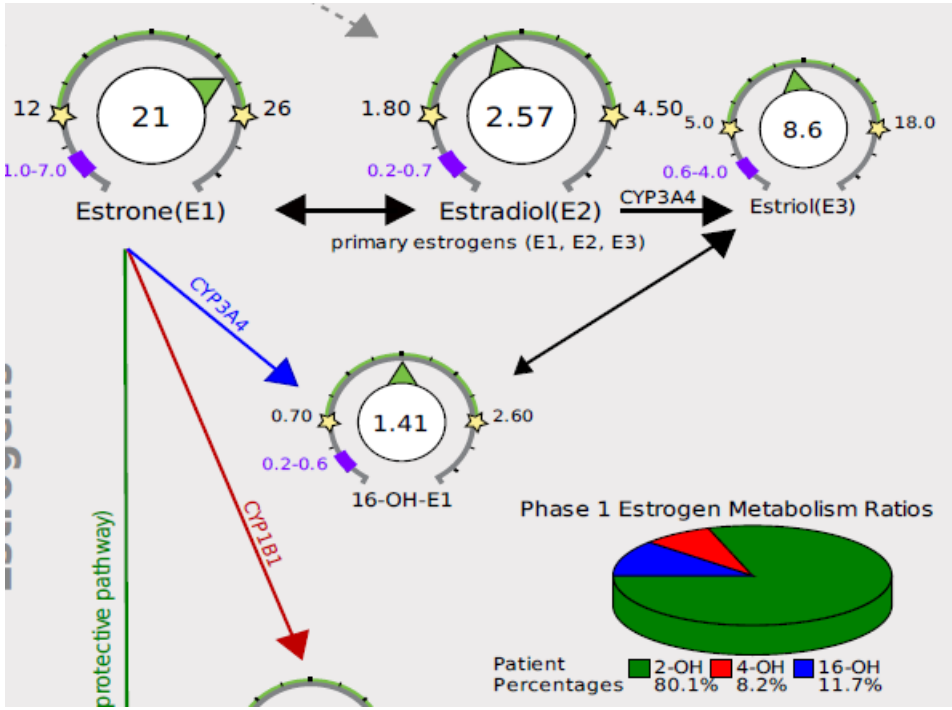


Moore SC, et al. J Natl Cancer Inst. 2016;108(10).

16OH Pathway

16OH Pathway

- 16OH estrogens are formed from CYP3A4 in the liver and in extrahepatic tissues:
  - Breast
  - Uterus
  - Placenta
  - Brain
- Binds and activates estrogen receptors
  - Some studies show a binding affinity that is greater than E2, but in others it may be more like E1.
- Described as:
  - Mitogenic (proliferative), pro-inflammatory, angiogenic
  - **Abnormal cell growth terrain**



Swaneck G, Fishman J. Proc Natl Acad Sci U S A. 1988;85.
Tsuchiya Y, et al. Cancer Lett. 2005;227(2):115-124.
Zhu BT, et al. Endocrinology. 2006;147(9):4132-4150.

16OH Pathway

- 16OH-E1 is associated with greater postmenopausal bone mineral density (BMD).
- This demonstrates that 16OH has estrogenic properties on the body's tissues.
- **Trying to increase the 16OH for BMD protection is not a proven pathway.**
  - **Estradiol, through postmenopausal HRT, is a proven pathway to bone protection and optimal metabolism is through the 2OH pathway.**
- If your patient is a healthy postmenopausal woman with low-risk factors but high 16OH, consider that interventions to lower it might feel like lowering estrogens (hot flashes, etc).

- 16OHE1 and E2 are high in pregnancy, which does not increase BC risk.
- While this pathway is associated with more overall estrogenic activity, keep in mind this isn't always bad.

Lim SK, et al. J Clin Endocrinol Metab. 1997;82(4):1001-1006.

2OH pathway

Back Again:

Moore et al. Shanghai Women's Health Study Cohort

399 postmenopausal invasive breast cancer patients

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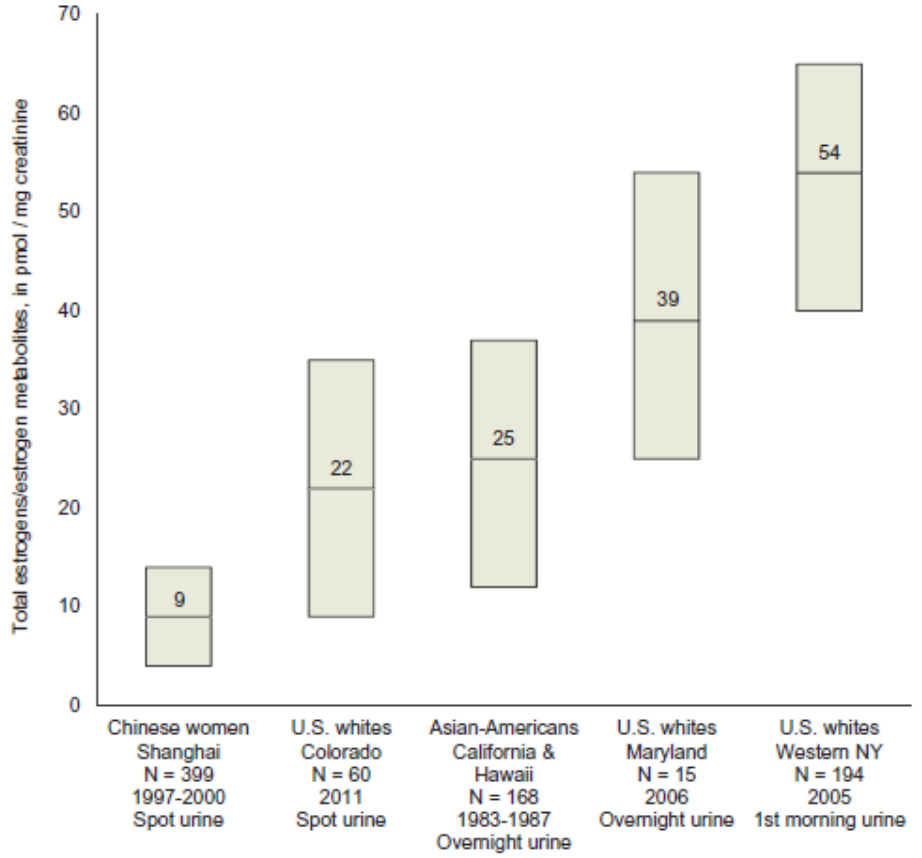


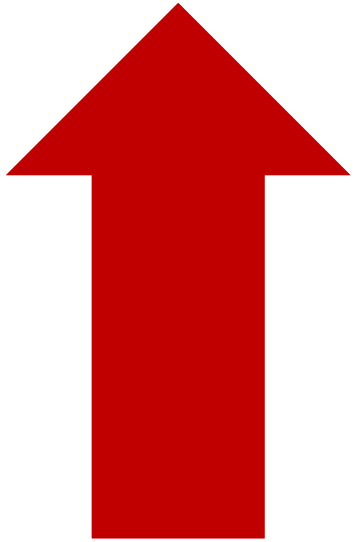
Moore SC, et al. J Natl Cancer Inst. 2016;108(10).

16OH Pathway

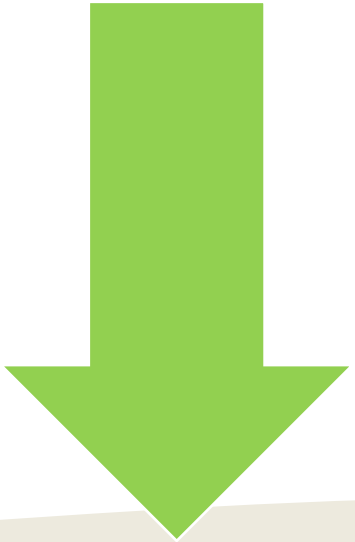
- Women's Health Initiative Study found reduced risk of BC in women who used Conjugated Equine Estrogens (CEEs) alone, but increased risk in women who used CEEs + Medroxyprogesterone Acetate (MPA), a synthetic progestin.

- They found that those in the CEE alone group had more extensive metabolism along the 2OH pathway and those using CEE+MPA had **more 16OH metabolism.**

- **Oral MPA** might upregulate the **16OH pathway.**
- **Keep in mind that BC incidence was rare with 6 additional cases per 10,000 users per year.**



CEE + MPA = Higher 16OH, Higher BC risk



CEE alone = Higher 2OH, Lower BC risk

Falk RT, et al. 2019;144(4):730-740.

16OH Pathway

Idiopathic Pulmonary Arterial Hypertension (iPAH):

- High estradiol is known to increase the risk and severity of idiopathic pulmonary arterial hypertension in men and postmenopausal women.
  - **Women are more likely** to get PAH than men.
- However, estradiol is also protective to the cardiovascular system, including in PAH.
  - **Women** with PAH have **higher survival rates than men**.
  - E2 inhibits inflammation in the cardiovascular tissues
- **Serum 16OH metabolites were higher in patients with iPAH.**
  - 16OH increases the expression of **inflammatory cytokines**
  - 16OH increases the growth and proliferation of blood vessels (**angiogenic**)
  - 16OH increases cellular division (**mitogenic/proliferative**)
  - Abnormal cell growth terrain

Denver N, et al. Pulm Circ. 2020;10(1):1-5.
Sun Y, et al. Front Cardiovasc Med. 2021;8.

16OH Pathway

Rheumatoid arthritis and systemic lupus erythematosus

- 16OH metabolites are significantly increased in the urine of patients with rheumatoid arthritis (RA) or systemic lupus erythematosus compared to controls.
- The SLE autoantibody binds to 16OHE1 metabolite- thus the 16OH metabolite may be stimulating this autoimmune process.
- Estrogenic activity is implicated in autoimmune disease, RA, and SLE.
- There is an **increased 16OH and decreased 2OH in patients with SLE and RA.**
- SLE and RA have both been associated with PAH (previous slide).

Khan WA. Adv Med Sci. 2019;64(1):72-78.
Lahita RG, et al. J Clin Endocrinol Metab. 1981;53(1):174-178.
Weidler C, et al. J Rheumatol. 2004;31(3):489-494.

16OH Pathway

Endometriosis

- CYP450 enzymes, including 1A1, 1B1, and 3A4 are present in endometrial tissue and are regulated by estrogen levels.
- CYP3A4 is increased in endometriotic lesions.
- CYP3A4 activity may be implicated in endometrial tissue growth, both entopic and ectopic.
- Increased CYP3A4 and 16OH may be mediated by environmental factors.
    - One possible mechanism is environmental stimulation of TGF-β and a mutation in Kruppel-like factor-11 (KLF11), which in turn alter 3A4 expression in endometrial tissue.
    - Mutations in KLF11 are seen with endometriosis.
    - May promote higher local 16OH and its negative effects.

Correa LF, et al. Endocrinology. 2016;157(9):3332-3343.
Zheng Y, et al. Endocrinology. 2014;155(11):4507-4520.

2:16 Ratio

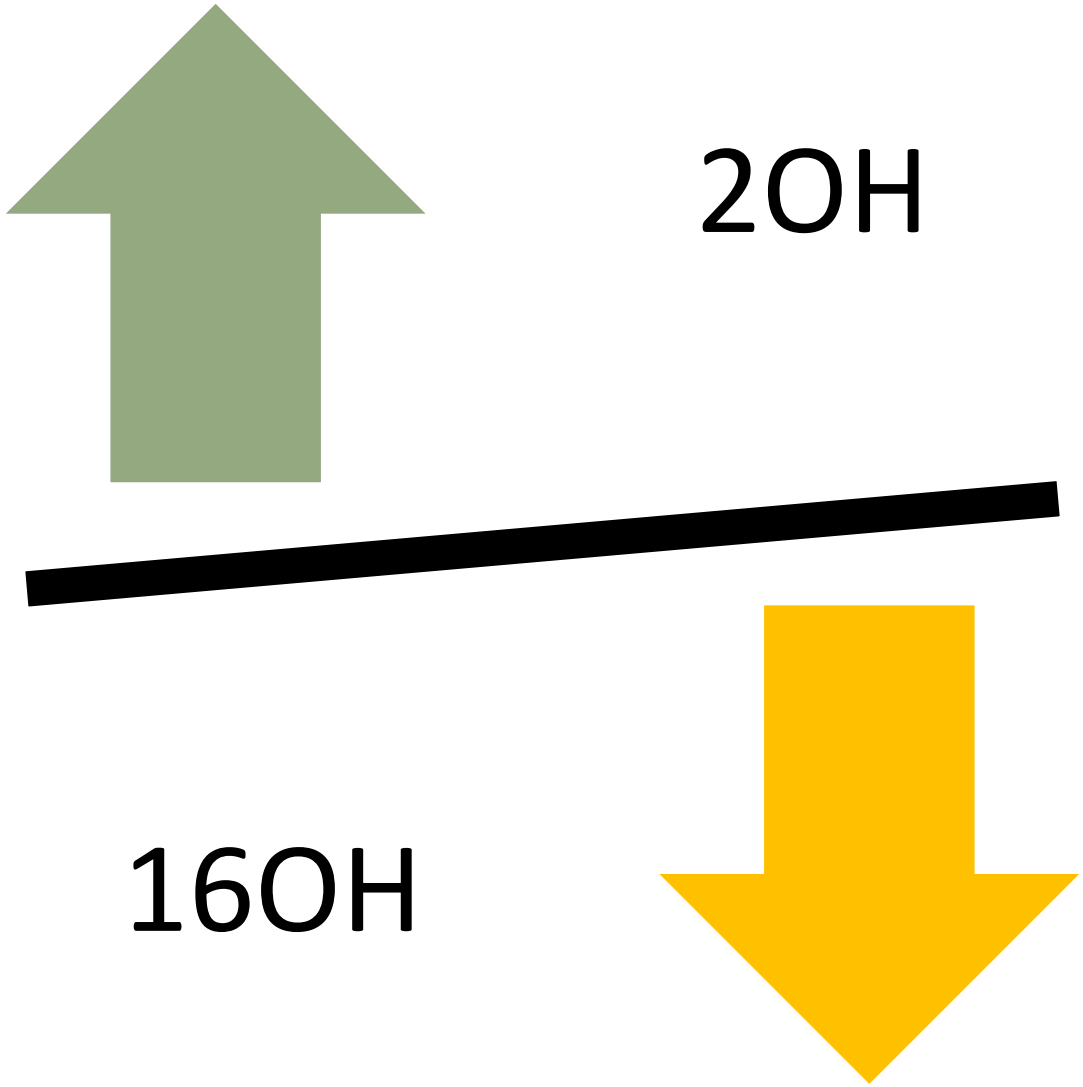
The 2:16 OH metabolite ratio helps understand the balance of these metabolites across a lifespan.

A higher ratio means that either 2OH is higher or 16OH is lower (or both).

A lower ratio means that 2OH is lower or 16OH is higher or both.

Studies find that a higher 2:16 ratio is better:

- Reduced BC risk
- Reduced All-cause mortality
- Normal BMI



Im A, et al. Carcinogenesis. 2009;30(9):1532-1535.

2:16 Ratio and BC Risk

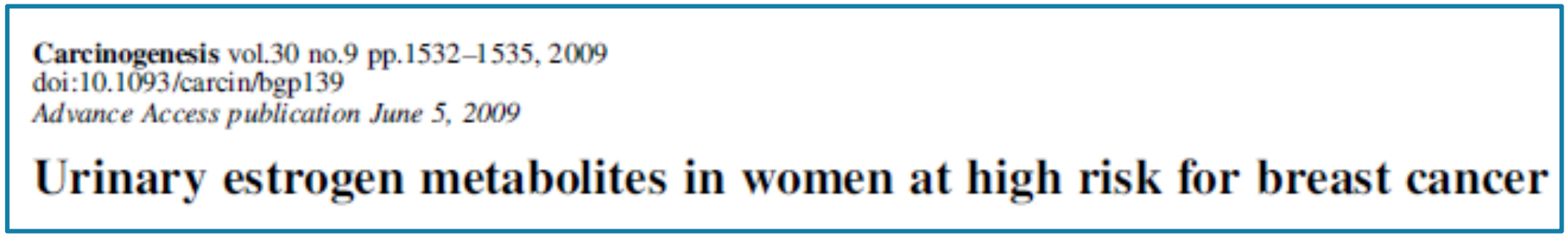
Urinary 2-OH-E1/16-OH-E1 ratio

Population/results:

77 high-risk women = Lower 2:16 ratio (meaning more 16OH or less 2OH)

30 BC patients = Lower 2:16 ratio (meaning more 16OH or less 2OH)

41 controls = Higher 2:16 ratio (meaning less 16OH or more 2OH)



Im A, et al. Carcinogenesis. 2009;30(9):1532-1535.

2:16 Ratio and BC Risk

GYNECOLOGIC ONCOLOGY

The ratio of the estradiol metabolites 2-hydroxyestrone (2-OHE1) and 16α-hydroxyestrone (16-OHE1) may predict breast cancer risk in postmenopausal but not in premenopausal women: two case–control studies

Xiangyan Ruan · Harald Seeger · Diethelm Wallwiener · Jens Huober · Alfred O. Mueck

2:16 ratio of estrone metabolites

2:16 ratio was significantly lower in postmenopausal BC patients than postmenopausal controls.

The 2:16 ratio was lower with higher BMI.

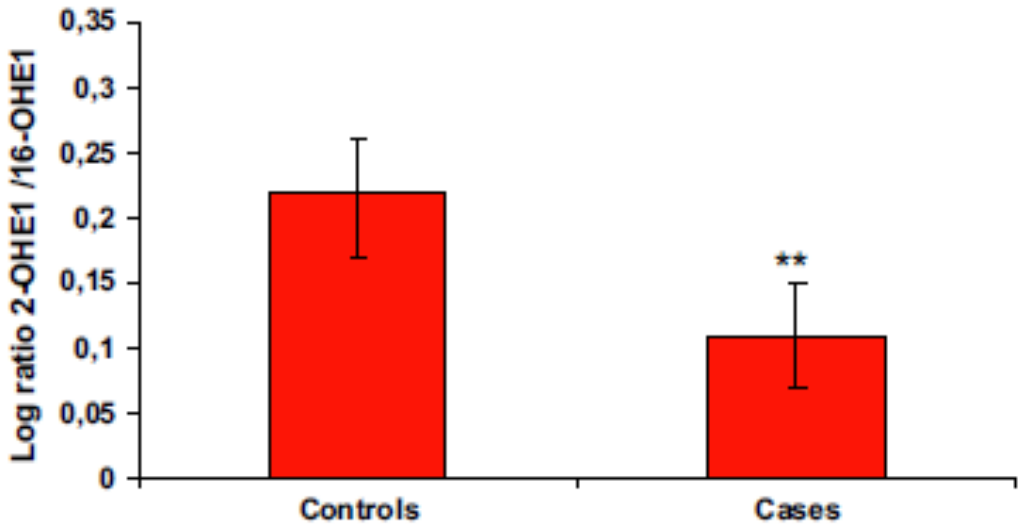


Fig. 2 Log ratio of 2-hydroxyestrone (2-OHE1) to 16α-hydroxyestrone (16-OHE1) in postmenopausal women without (controls $N = 206$) and with (cases $N = 207$) breast cancer. (means, 95 % CI; $^{**}p < 0.01$ vs. controls)

Ruan X, et al. Arch Gynecol Obstet. 2015;291(5):1141-1146.

2:16 Ratio and Mortality

Population based study:

Urine samples collected after BC diagnosis (1996-97).

Mean 17.7 years follow-up.

683 BC patients and 434 age-matched controls.

2:16 ratio was inversely associated with all-cause mortality, especially among women with BC.

Higher 2:16 ratio showed lower risk of BC and CVD mortality.

Urinary Estrogen Metabolites and Long-Term Mortality Following Breast Cancer

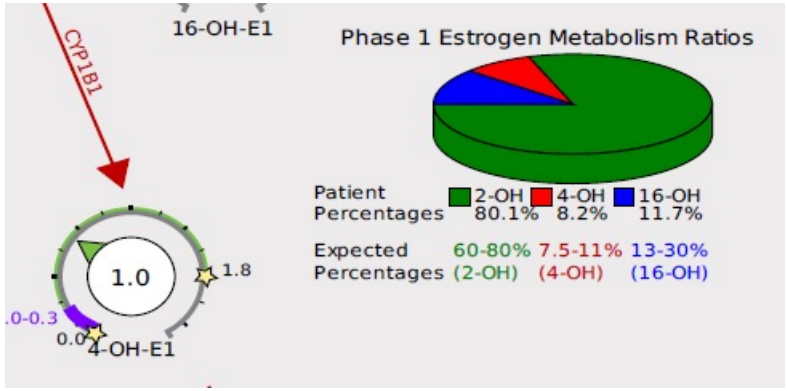
Tengteng Wang, PhD,[1,2,3,*] Hazel B. Nichols, PhD,[1] Sarah J. Nyante, PhD,[1,4] Patrick T. Bradshaw, PhD,[5] Patricia G. Moorman, PhD,[6] Geoffrey C. Kabat, PhD,[7] Humberto Parada Jr, PhD,[8] Nikhil K. Khankari, PhD,[9] Susan L. Teitelbaum, PhD,[10] Mary Beth Terry, PhD,[11] Regina M. Santella, PhD,[12] Alfred I. Neugut, MD, PhD,[11,13] Marilie D. Gammon, PhD[1]

Wang T, et al. JNCI Cancer Spectr. 2020;4(3):pkaa014.

4OH Pathway

4OH Pathway

- 4OH metabolites are formed by the CYP1B1 enzyme:
  - Liver
  - Breast
  - Uterus
- 4OH has some binding affinity for the ER
  - Mixed reports: some say it has binding affinity, some say none
- 4-OH-E1 is **unstable** and forms **“reactive”**, or oxidative quinones.
- Oxidative quinones can bind to DNA and **cause DNA damage.**
- 4-OH-E1 is **slower** to go through phase II **methylation** than 2-OH-E1.



Liehr JG, Ricci MJ. Proceedings of the National Academy of Sciences. 1996;93(8):3294-3296.
Tsuchiya Y, et al. Cancer Lett. 2005;227(2):115-124.

4OH Pathway

Liehr et al. 1996 found that mammary adenocarcinoma and fibroadenomas had significantly greater 4OH metabolites than normal breast tissue.

Graph:

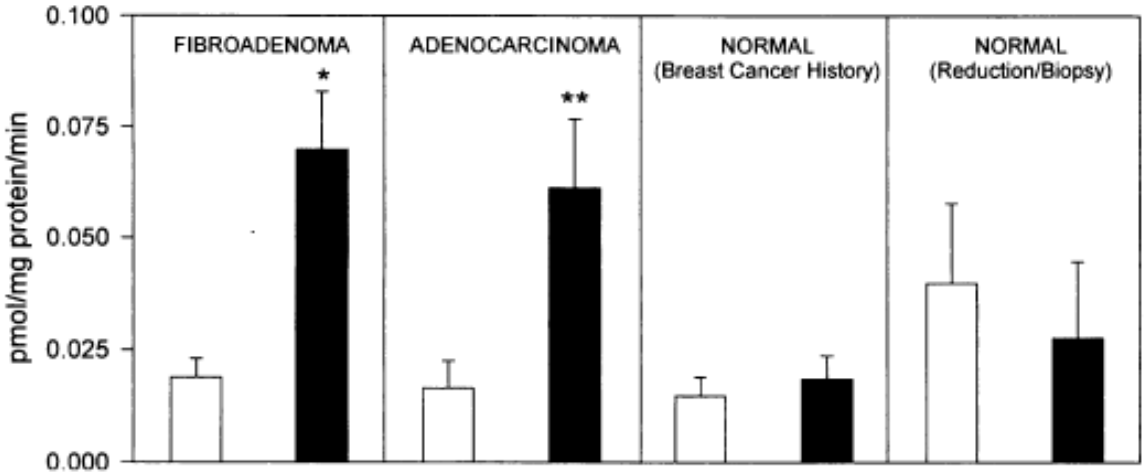
White bars show 2OHE2

Black bars show 4OHE2

4-Hydroxylation of estrogens as marker of human mammary tumors

(estrogen metabolism/catecholestrogen formation/2-hydroxyestradiol/4-hydroxyestradiol/human breast tumors)

JOACHIM G. LIEHR* AND MARY JO RICCI



Liehr JG, Ricci MJ. Proceedings of the National Academy of Sciences. 1996;93(8):3294-3296.

4OH Pathway

Estrogen Metabolism and Risk of Breast Cancer in Postmenopausal Women

Barbara J. Fuhrman, Catherine Schairer, Mitchell H. Gail, Jennifer Boyd-Morin, Xia Xu, Laura Y. Sue, Saundra S. Buys, Claudine Isaacs, Larry K. Keefer, Timothy D. Veenstra, Christine D. Berg, Robert N. Hoover, Regina G. Ziegler

Manuscript received January 19, 2011; revised July 16, 2011; accepted December 2, 2011.

Fuhrman et al. 2012 found an increased risk of invasive breast cancer in:

- Higher unconjugated serum Estradiol (**active estradiol**)
- 4OH/4Me (**more 4OH**, slow methylation of 4OH)

- 2OH metabolism was associated with **lower risk**

4OH Pathway

- **4OH metabolites were increased in women with BC compared to controls.**
- 4OH metabolites induced **malignant transformation and tumorigenesis** in nude mice.
- 4OH metabolites **compromised spindle-assembly checkpoints**, one possible mechanism for carcinogenesis.
- Transgenic mice who had genetically increased CYP1B1 expression **developed mammary cancer.**

Among many alterations of EMs in the breast cancer group, the most significant one in this study was an increase in 4-hydroxy metabolites. Urine 4-OH-E_1 in the patients with breast cancer was three times higher than that in healthy women, while other EMs changed less. The best indicator that reflected the risk of breast cancer was the ratio of 4-hydroxy metabolites to total estrogen. The findings of this study were consistent with the assumption that the metabolic conversion of E_2 to 4-OH-E_2 and its additional activation to reactive semiquinone/quinone intermediates may be genotoxic, leading to various types of DNA damage, and have a direct role as tumor initiators [15-20].

Miao S, et al. Am J Transl Res. 2019;11(8):4992-5007.

4OH Pathway

- Urine samples + Lung tissue samples from healthy controls and small cell lung cancer patients.
  - **4OH metabolites** were **higher in tumors** versus normal lung tissue.
  - **4OH metabolites** were **higher in the urine** of small cell lung cancer patients compared to controls.

Research Paper

Estrogen metabolism in the human lung: impact of tumorigenesis, smoke, sex and race/ethnicity

Jing Peng[1], Sibele I. Meireles[1], Xia Xu[2], William E. Smith[3], Michael J. Slifker[1], Stacy L. Riel[1], Shumenghui Zhai[4], Guo Zhang[4], Xiang Ma[4], Mindy S. Kurzer[3], Grace X. Ma[4] and Margie L. Clapper[1]

4OH Pathway

- Breastcancer.org article about BPA and BC risk:
  - BPA acts as a weak, synthetic estrogen.
  - It is found in rigid plastic product containers and linings or food containers, receipts.
  - **May increase risk of some cancers**.

- In a 2014 study of urinary estrogen metabolites and BPA in Korean adults, higher BPA was associated with **higher 4OH metabolites and parent estrogens.**

https://www.breastcancer.org/risk/risk-factors/exposure-to-chemicals-in-plastic
Kim EJ, et al. Sci Total Environ. 2014;470-471:1401-1407.

Phase II
COMT Methylation

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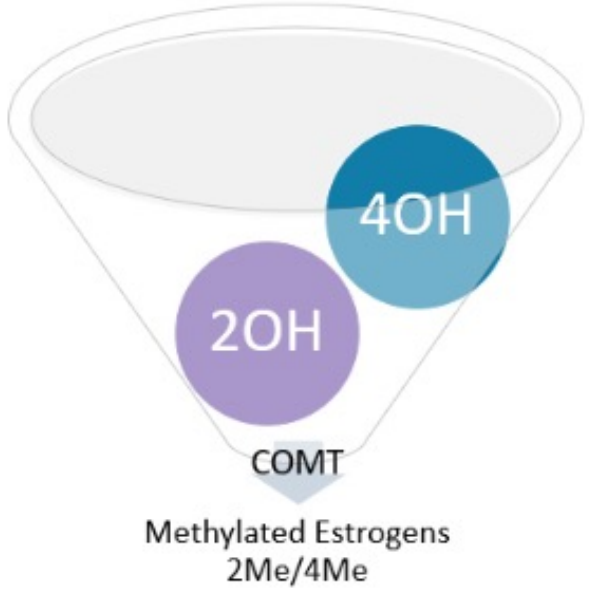
COMT Enzyme

- Low COMT activity has been associated with increased catechol estrogens.
  - In effect, “backing up” the pathway and leaving the oxidative phase I metabolites to form reactive quinones
- Slow COMT activity has been linked to:
  - Endometriosis
  - Endometrial cancer
  - Breast cancer
  - Uterine fibroids (mixed data)
- COMT deactivates catechol estrogens (2- and 4OH metabolites).
  - By deactivating them, it prevents them from causing DNA damage.
  - If COMT is not working well, we may have build-up of 2- and 4-OH estrogens and their negative effects.



de Oliveira E, et al. Maturitas. 2008;60(3-4):235-238.
Samavat H, et al. Cancer Lett. 2015;356(2 Pt A):231-243.
Zhu BT, et al. J Biol Chem. 1996;271(3):1357-1363.

COMT Enzyme

- The COMT enzyme has some significant polymorphisms that have been associated with disease states.
    - The **Val158Met polymorphism** is associated with **slower** activity than the wild type.
    - A meta-analysis of studies looking at COMT enzyme polymorphisms found that the **Val158Met polymorphism is associated with breast cancer susceptibility in Asian women**.

Rai V, et al. Asian Pac J Cancer Prev. 2017;18(5):1243-1250.

COMT Enzyme

- Several studies on COMT genetics and **uterine fibroids** (leiomyomas) have found the **COMT Val158Met polymorphism is associated with an increased risk of developing uterine fibroids**.
- A meta-analysis by Alset et al. in 2022 looking at the Val158Met polymorphism found it is **protective** for uterine fibroid risk.
  - A study found that green tea extract protects against and can shrink uterine leiomyomas in part via **reduced COMT activity**.
- A 2014 study found **uterine fibroids increased with CYP1A1, 1B1, and COMT polymorphisms** that impacted estrogen metabolism.
- At this point **the literature is mixed** about COMT activity and uterine leiomyomas.

Alset D, et al. Reprod Sci. 2022.
Ates O, et al. Mol Cell Biochem. 2013;375(1-2):179-183.
de Oliveira E, et al. Maturitas. 2008;60(3-4):235-238.
Zhang D, et al. Gynecol Obstet Invest. 2014;78(2):109-118.

COMT Enzyme

- The COMT enzyme function is an important final step in estrogen detoxification.
- Slowed estrogen methylation has been associated with **increased oxidative metabolites:**
  - 2OHE1/E2
  - 4OHE1/E2
- However, **green tea extract slows COMT** activity and is associated with health benefits, including reduced BC risk.
  - This is likely because it is a **powerful antioxidant**, neutralizing the negative impact of 4OH and sometimes even 2OH metabolites.
- Fast COMT activity has not been associated with estrogen-related health issues.
- Slow COMT activity may not be a serious risk if the patient is healthy and has **good antioxidant status**.

Fuhrman BJ, et al. Nutrition Journal. 2013;12(1):25.

Estrogen Metabolism Overview

Overview of Estrogen Metabolism

CYP1A1/1A2 (Major)	CYP1B1	CYP3A4	COMT
Healthy	“Estrogenic”, DNA Damage, ROS	“Estrogenic”	Inhibits carcinogenesis
2OH-E1/E2 (Catechols)	4OH-E1/E2 (Catechols)	16OH-E1/E2	2Me-E1/E2, 4Me-E1/E2
Liver, target tissues	Liver, target tissues	Liver, target tissues	Liver, target tissues
Low ER binding affinity, anti-estrogenic impact	Possible ER binding and activation	Moderate to high ER binding affinity and activation	Low to no ER binding affinity, anti-estrogenic impact
Oxidative, but stable	Oxidative, unstable	Oxidative, stable	Stable
Non-estrogenic, anti-estrogenic activity. Inhibit cell growth and proliferation.	Estrogenic, Genotoxic,	Inflammatory, mitogenic, angiogenic, proliferative	Non-estrogenic, anti-carcinogenic activity. Inhibit cell growth and proliferation.
Rapid clearance by COMT enzyme (phase II)	Slower clearance by COMT enzyme (phase II)	Sulfated	COMT mediated, genetic variants impact function

What Affects Estrogen Detox?

- May impair healthy detox:
  - Alcohol consumption
  - Dysbiosis
  - Low-fiber diet
  - Sedentary lifestyle
  - Tobacco use
  - Obesity
- May support healthy detox:
  - **Sulforaphane**
  - **Cruciferous vegetables**
  - **Resveratrol**
  - Glutathione
  - Green tea
  - **Rosemary**

Highlighted in blue = promotes greater 16OH metabolism
Highlighted in red = promotes greater 4-OH metabolism
Highlighted **in green**= promotes greater 2OH metabolism

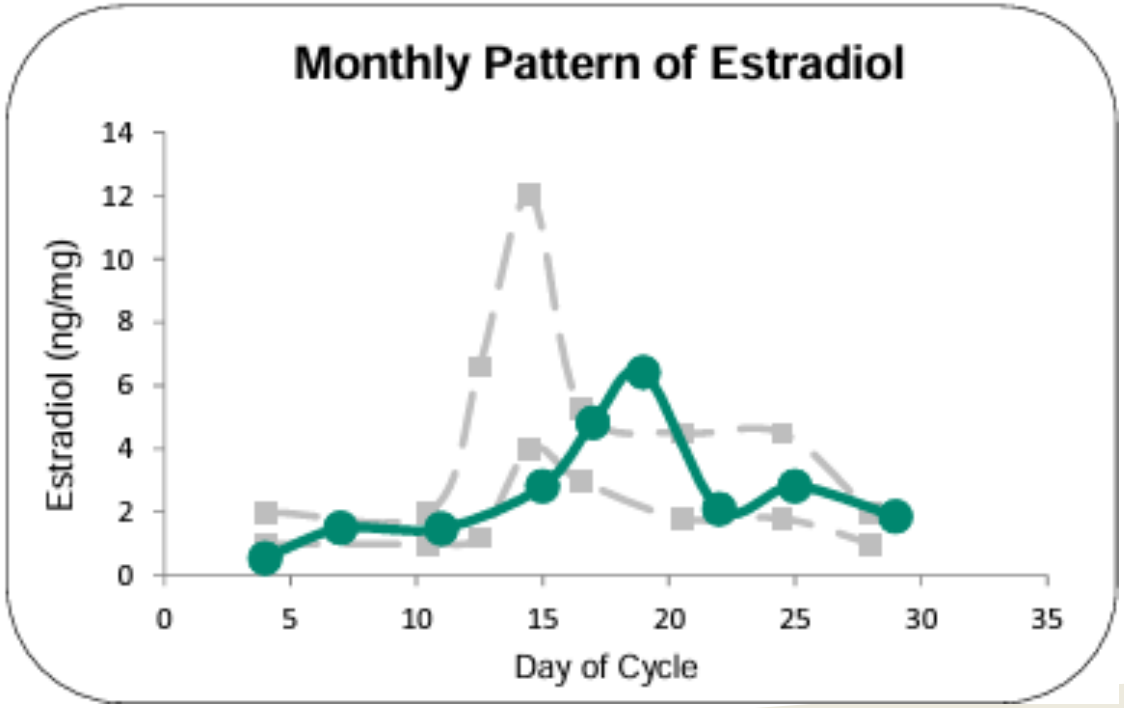
Hodges RE, Minich DM. J Nutr Metab. 2015;2015:1-23.

How do we measure estrogen metabolism?

How Do We Measure Estrogen Metabolism?

Estrogen metabolism is commonly measured in ratios due to:

- Large variation in estrogen levels.
- Ratios help to understand which metabolite is more prevalent, regardless of levels.



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How Do We Measure Estrogen Metabolism?

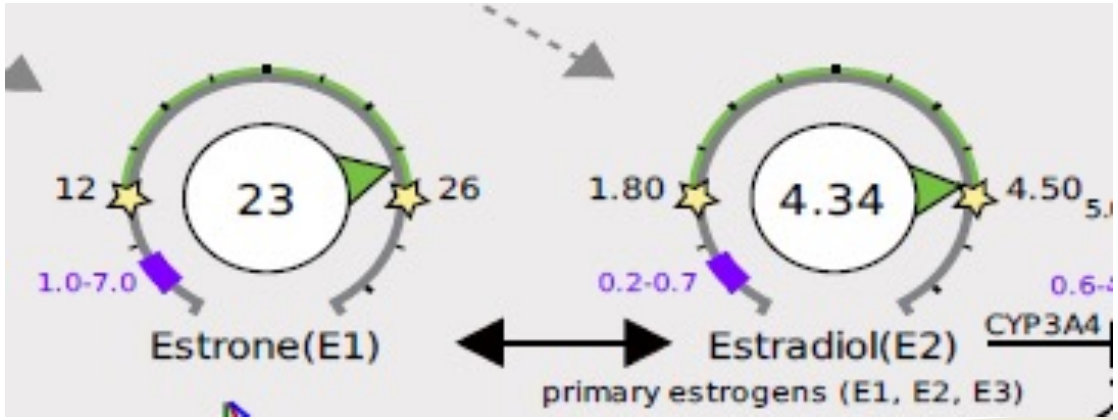
Estrogen metabolism is commonly measured in ratios.

- Metabolite actions are **not isolated.**
  - Since the metabolites are formed in the liver and target tissues, it is best to review their actions in a relationship or **ratio.**
  - For example, 2-OH is anti-proliferative and 16-OH is proliferative, therefore a ratio that shows what metabolite dominates is helpful.

- Keep in mind that if 4OH or 16OH are high, treatment should be considered even if the ratio is good.

How Do We Measure Estrogen Metabolism?

- Estrogen metabolism ratios should use Estrone Metabolites.
  - Estrone is more abundant at all stages of life.
  - Because E1 metabolites are more abundant, the ratios of E1 are easier to measure (with LC-MS/MS) and produce more stable ratios.
    - This is especially true in menopause.
  - **Stable ratios are more clinically reliable**.
  - Much of the research we have looked at follows estrone metabolism due to its abundance and it is a feature of studies on estrogen metabolism and with clinical impacts.
  - DUTCH has found **E2 metabolism ratios vary more day to day than E1 metabolism,** therefore E1 is more clinically reliable especially when basing treatment plans from a one-day test.



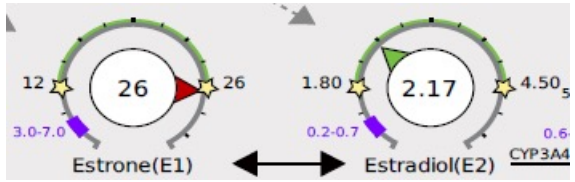
How Do We Measure Estrogen Metabolism?

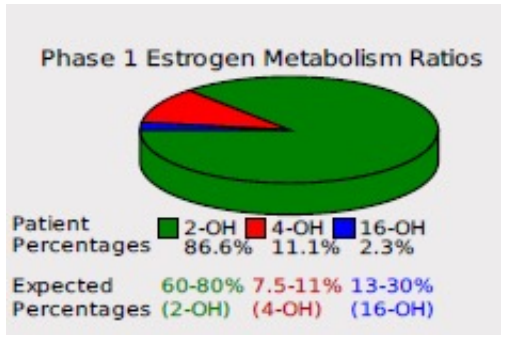
The steps to measuring estrogen metabolism:

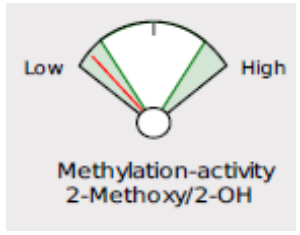
- Review parent estrogens
- Follow estrone metabolism
- Review population ranges for metabolite levels
- Review metabolite ratios

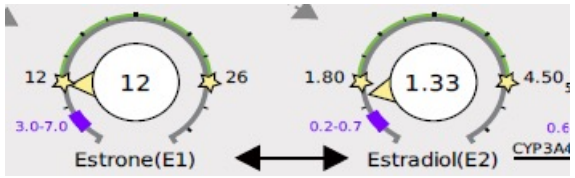
DUTCH Examples

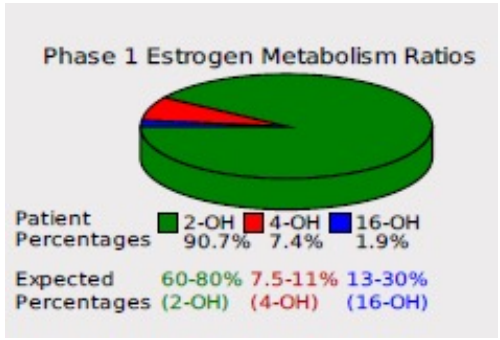
DUTCH Estrogen Metabolism Testing

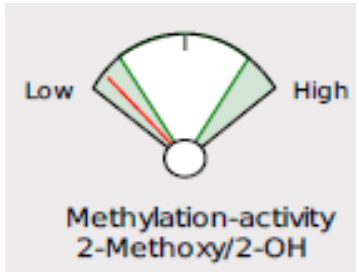


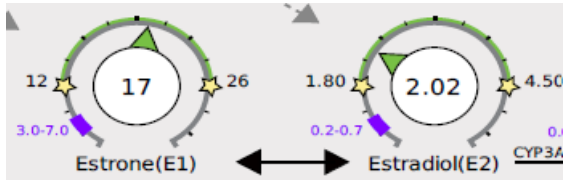


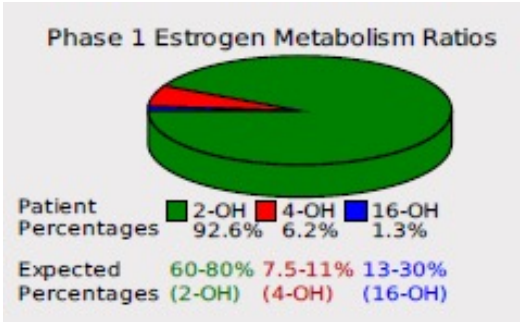


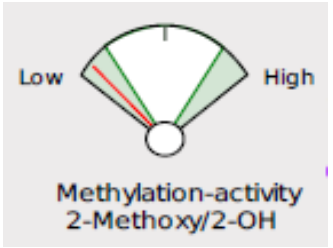












Example: 39-year-old female Cycle Map Estrogen metabolism.

Estrogen levels fluctuate, and **metabolism ratios are stable**.

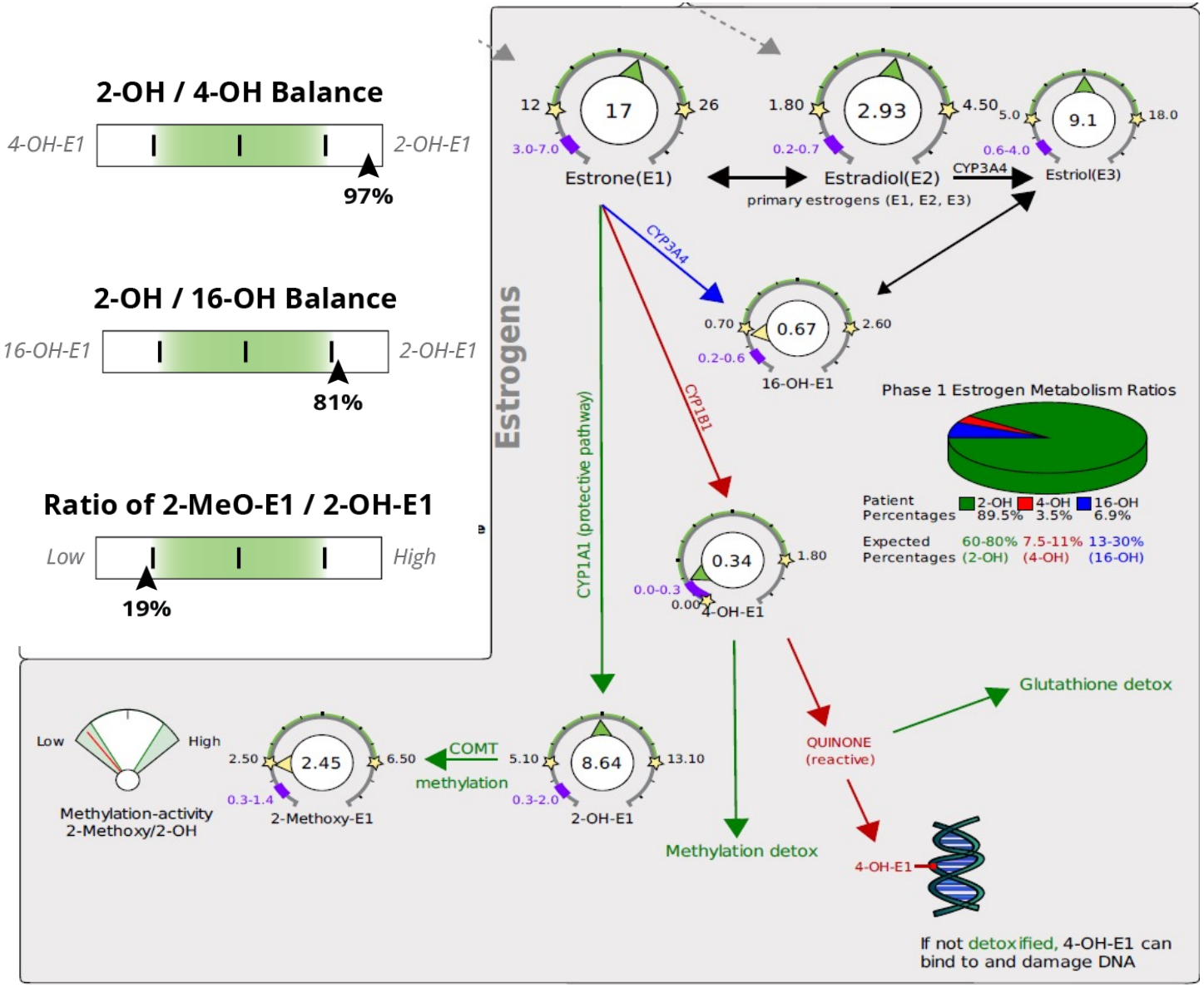
Methylation is poor.

Normal Phase I, Slow Phase II

Premenopausal 41-year-old female

Does not report significant estrogen related symptoms

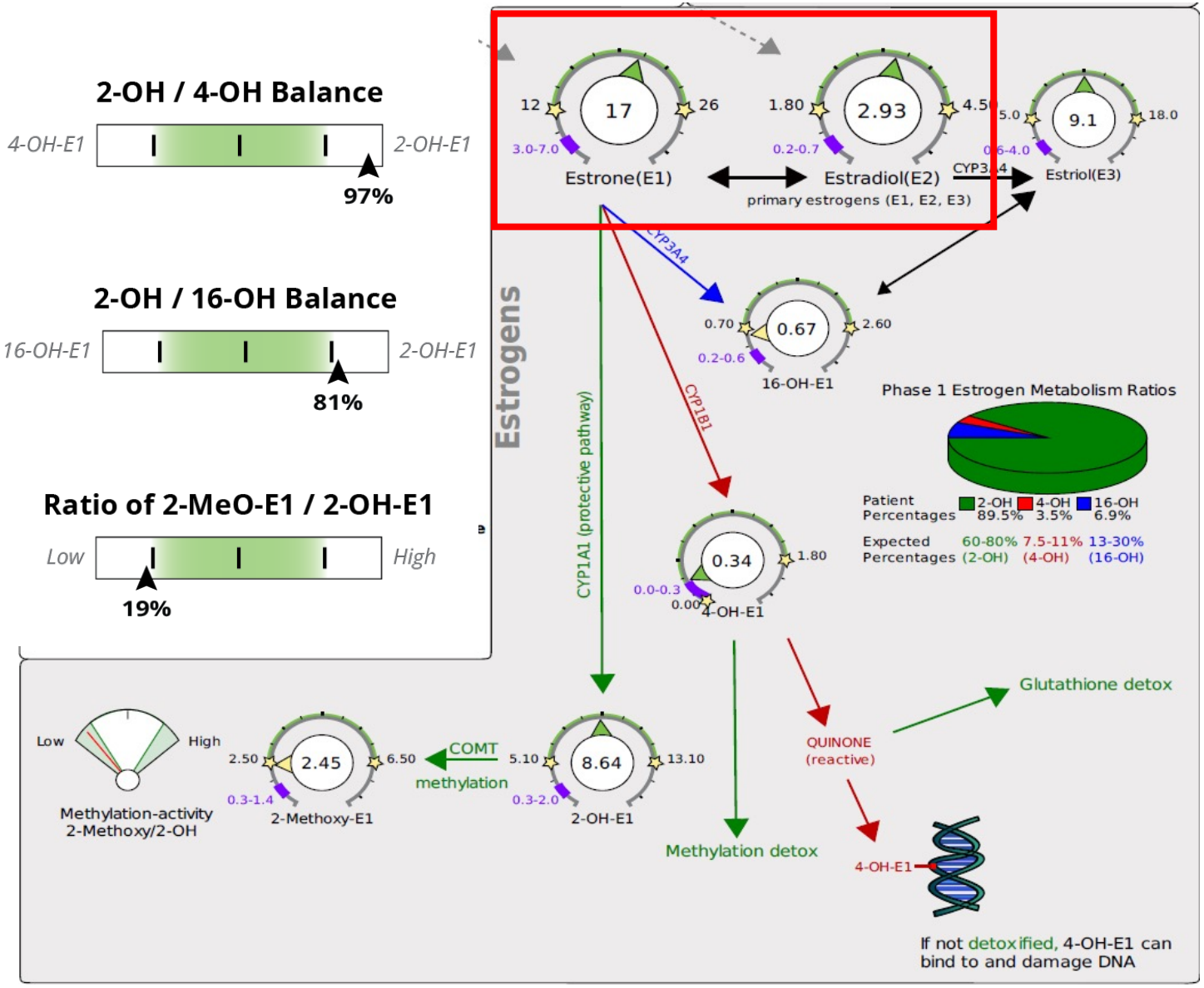
(Testing appears to be centered around adrenal and androgen concerns)



Normal Phase I, Slow Phase II

Premenopausal 41-year-old female

Parent estrogens are in the normal range.



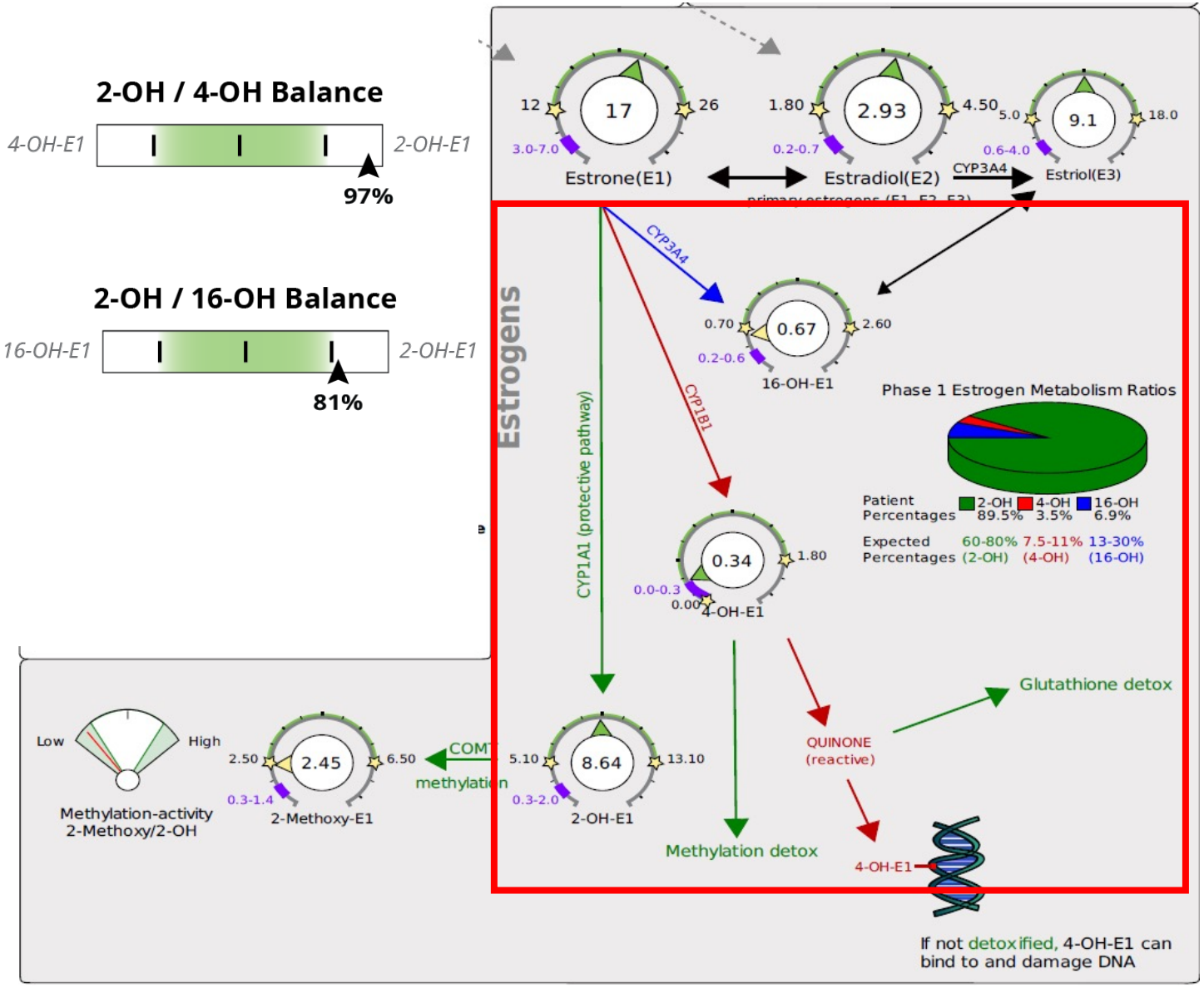
Normal Phase I, Slow Phase II

Premenopausal 41 year old female

Phase I dials are in the normal range:

Remember, low 16 is not “bad”

The pie chart is dominated by the green, healthy pathway.



Normal Phase I, Slow Phase II

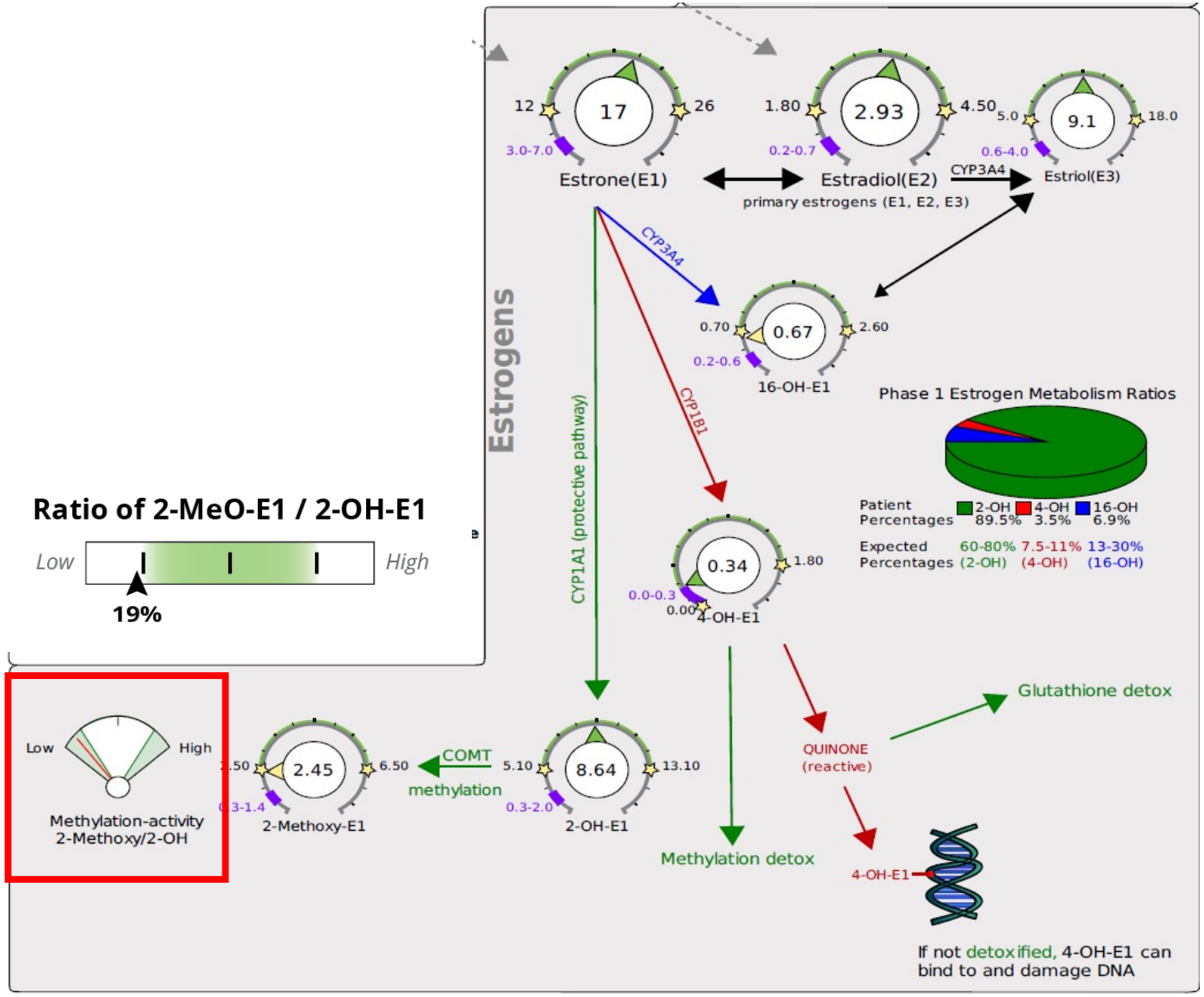
Premenopausal 41 year old female

Her COMT ratio is slow.

This does not appear to be causing too much 4OH buildup.

If this were your case, consider COMT support:

- Magnesium
- B6, B12, Folate
- Choline, methionine, TMG



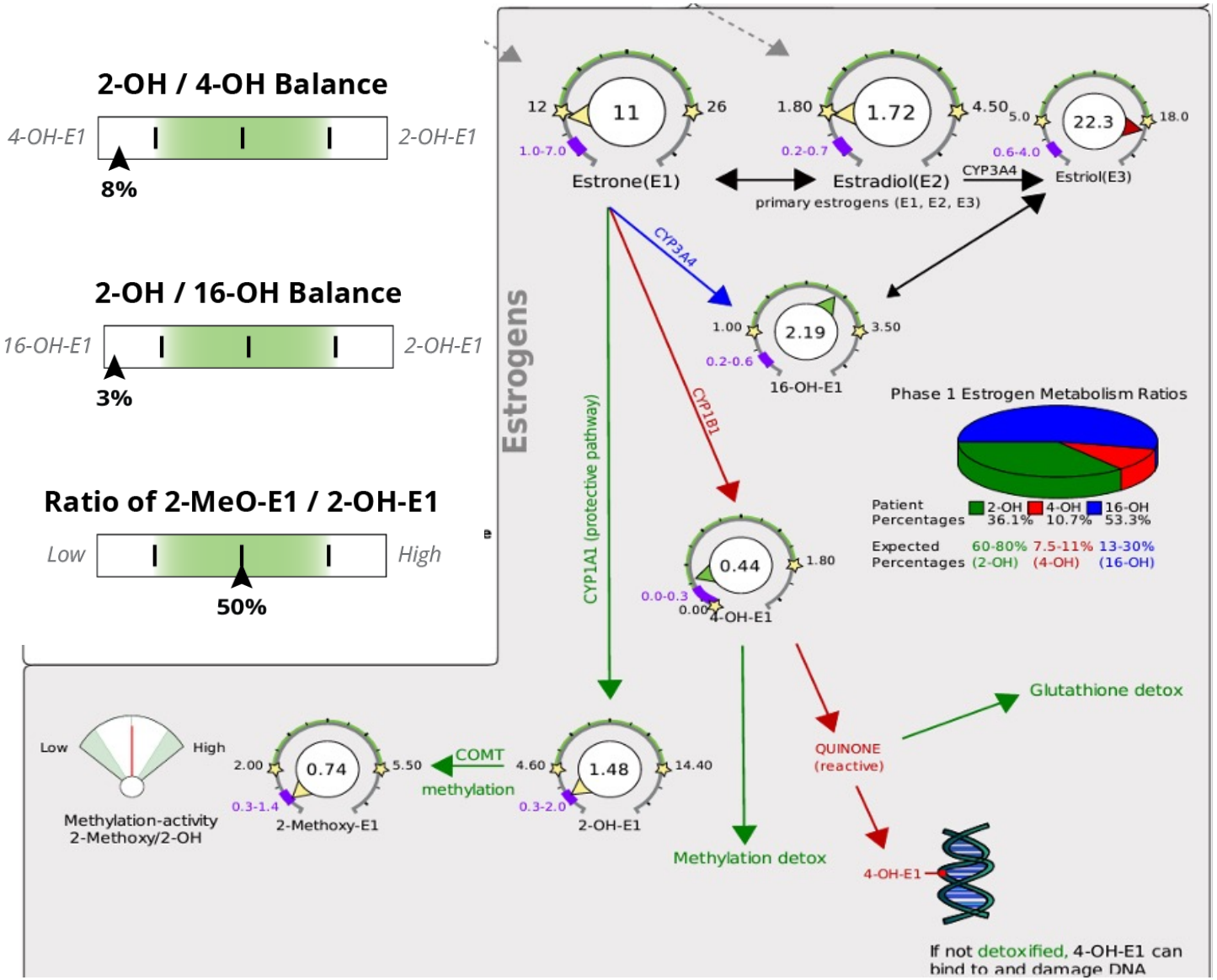
Normal Phase I, Slow Phase II

Case Points:

- COMT also methylates catecholamines, such as dopamine.
- If slow, it may be associated with anxiety and difficulty sleeping.

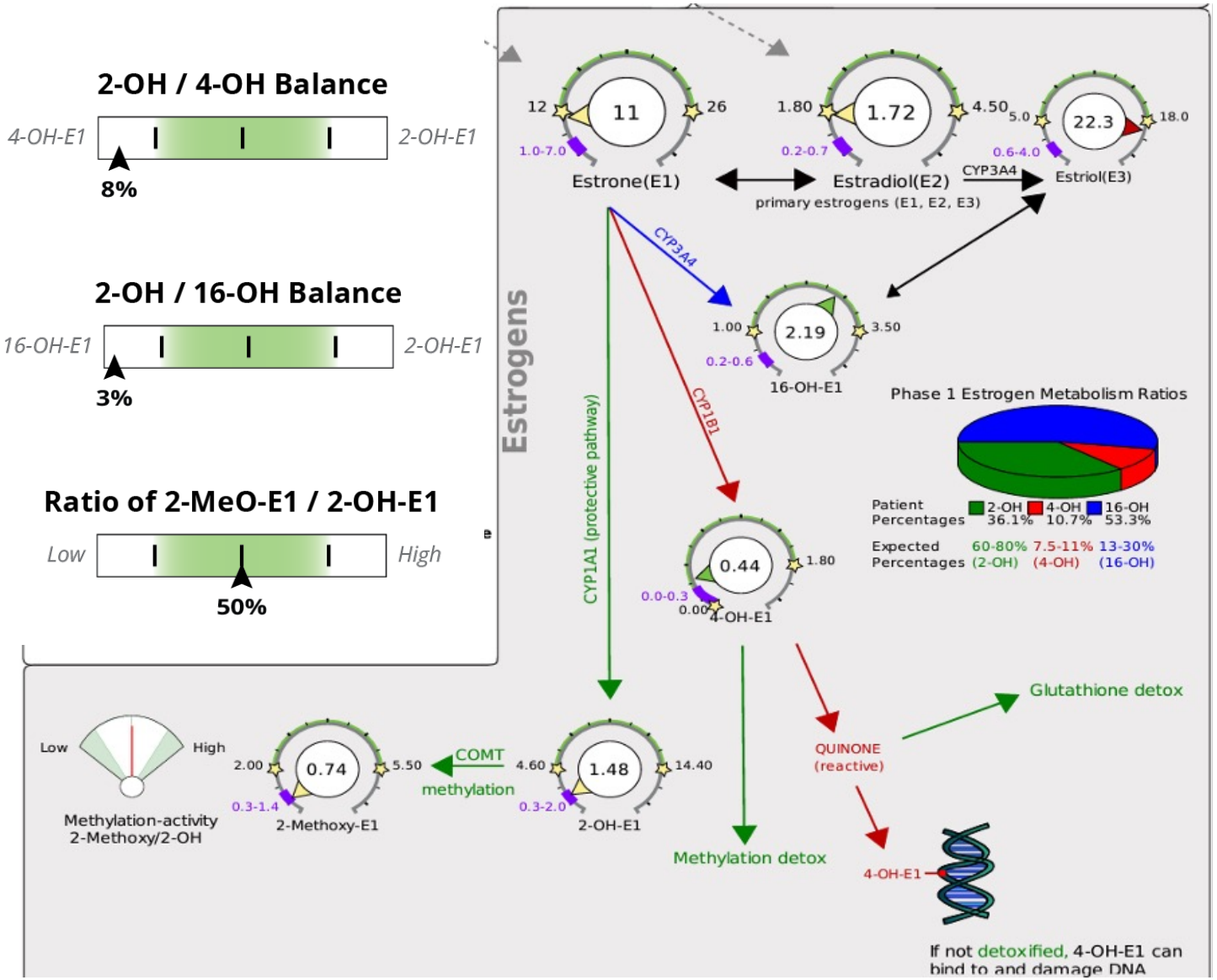
16OH Preference, Normal phase II, Endometriosis case

- 28-year-old cycling female with endometriosis
- Excellent diet and lifestyle.
- Whole 30 style diet, low red meat, lots of plant foods, pilates instructor.
- Significant endometrial pain with her menses.
- The pie chart reveals a strong 16OH preference which we know is estrogenic.
  - Her strong 16 preference obscures that she also has a strong 4OH preference, which we can see if we review the 2:4 separately.



Low 2OH, Normal phase II, Endometriosis case

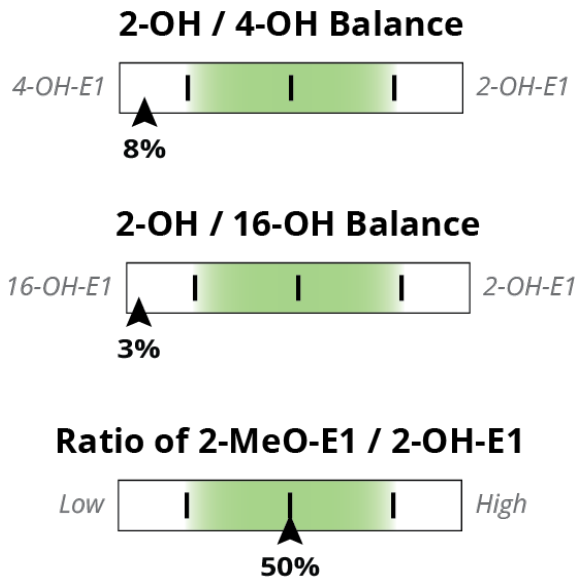
- This initial report was treated with DIM to support the 1A1 pathway.
- DIM is only used if lowering E1 and E2 is a good idea.
- DIM reduced the 16-OH metabolism significantly, along with greatly reducing her endometriosis pain.
- DIM introduced a new symptom: vaginal dryness.
- We removed the DIM and focused on ground flax, organic soy foods, and a sulforaphane supplement.
- Endometriosis pain remained improved, while vaginal symptoms returned to normal after removing the DIM.

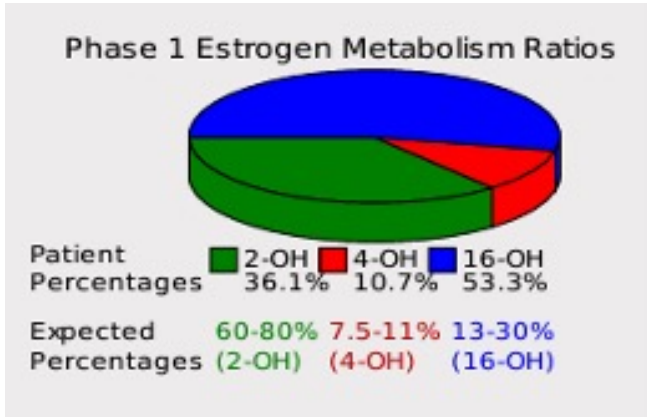


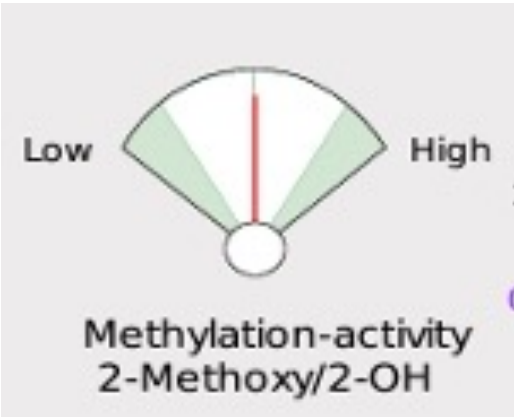
Low 2OH, Normal phase II, Endometriosis case

Endometriosis Case Points:

- Looking at 2OH:4OH independently was helpful to see it was also out of balance.
- 16OH may be associated with estrogenic symptoms even if the parent estrogens aren't high.
- DIM powerfully activates the 2OH pathway, which can lower estrogens.
  - This may not always be desirable.







4OH preference, Fast Phase II Methylation

52-year-old postmenopausal female with hot flashes, night sweats, and vaginal dryness.

High relative (pie chart, slider) 4OH metabolites.

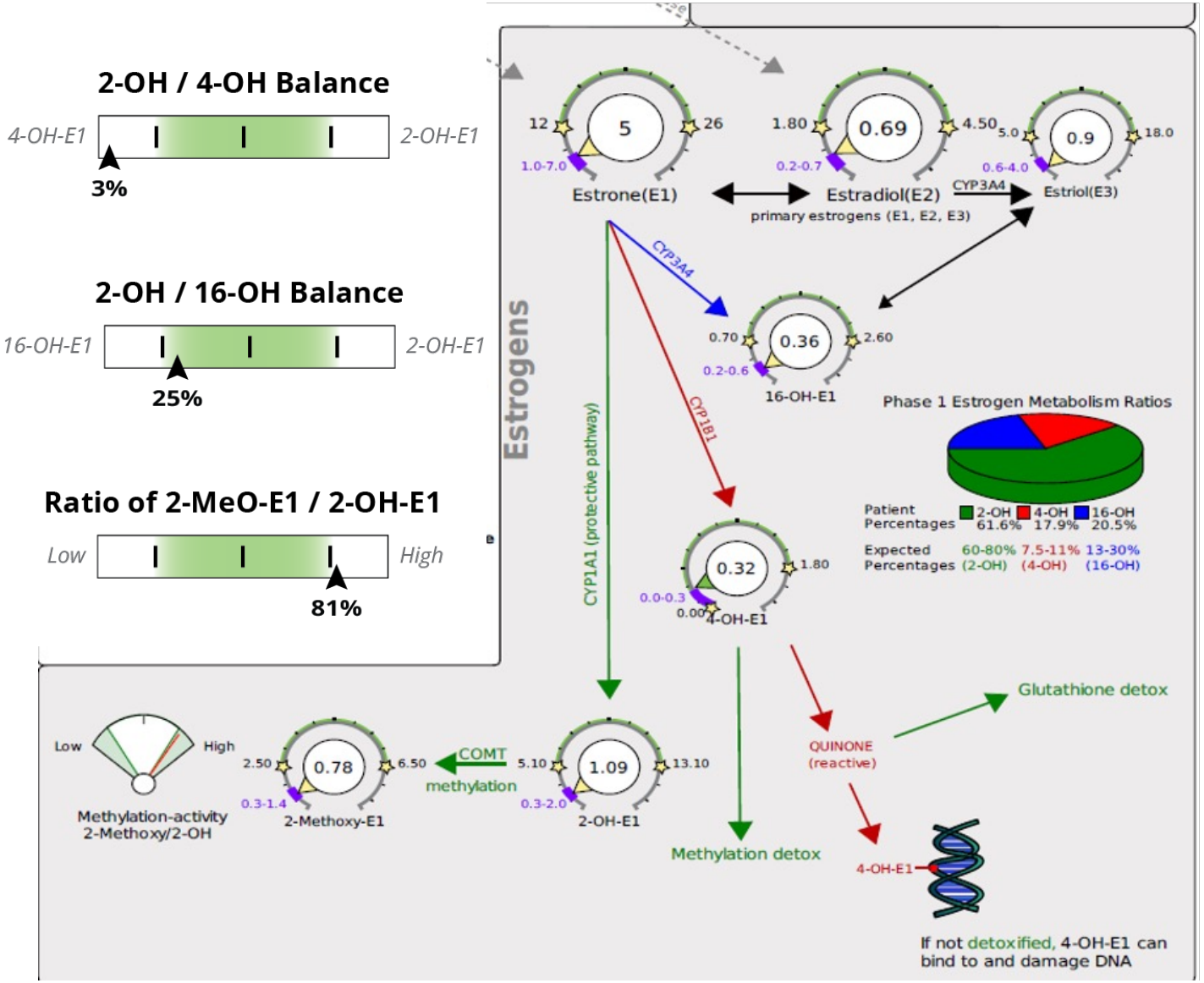
Methylation is fast.

Although the actual 4-OH is not very high, compared to some premenopausal females, in a relative sense, it is high.

This is especially concerning if she increases her estrogens using HRT.

Because methylation is fast (which is good), supporting the 2-OH and considering glutathione support may reduce her overall risk.

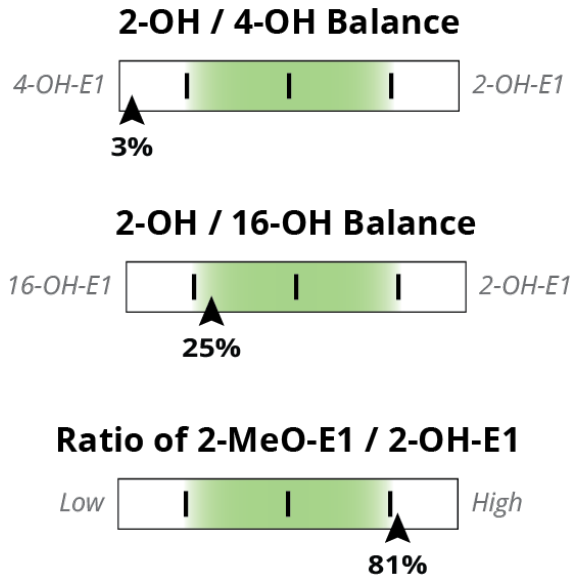
Consider supporting the 1A1 pathway with sulforaphane and N-Acetyl-Cysteine or GSH support, especially if going on HRT.

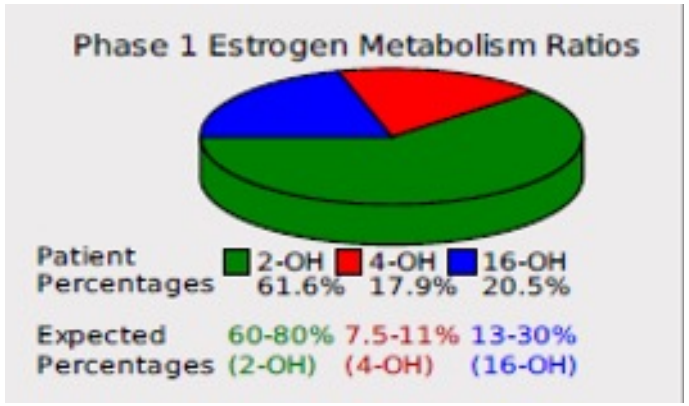


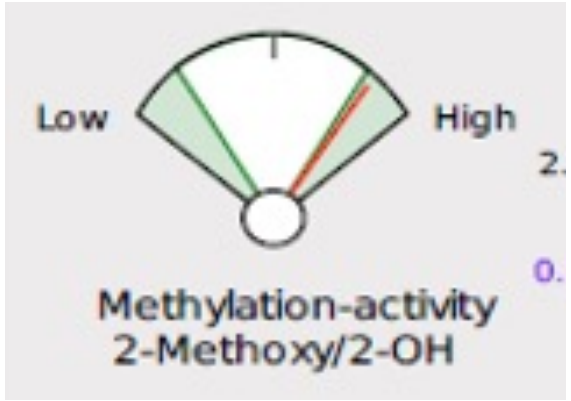
4OH preference, Fast Phase II Methylation

Postmenopausal HRT candidate Case Points:

- Consider the potential of low metabolites to cause harm in a postmenopausal patient when considering approaches to reading estrogen metabolism patterns.
- If parent estrogens and metabolite levels are low but ratios are not good:
  - Consider the impact of increasing estrogens (such as with HRT)
  - Consider the impact of DIM on low estrogen symptoms
  - Consider overall health, risk factors, and antioxidant status.







2OH Low

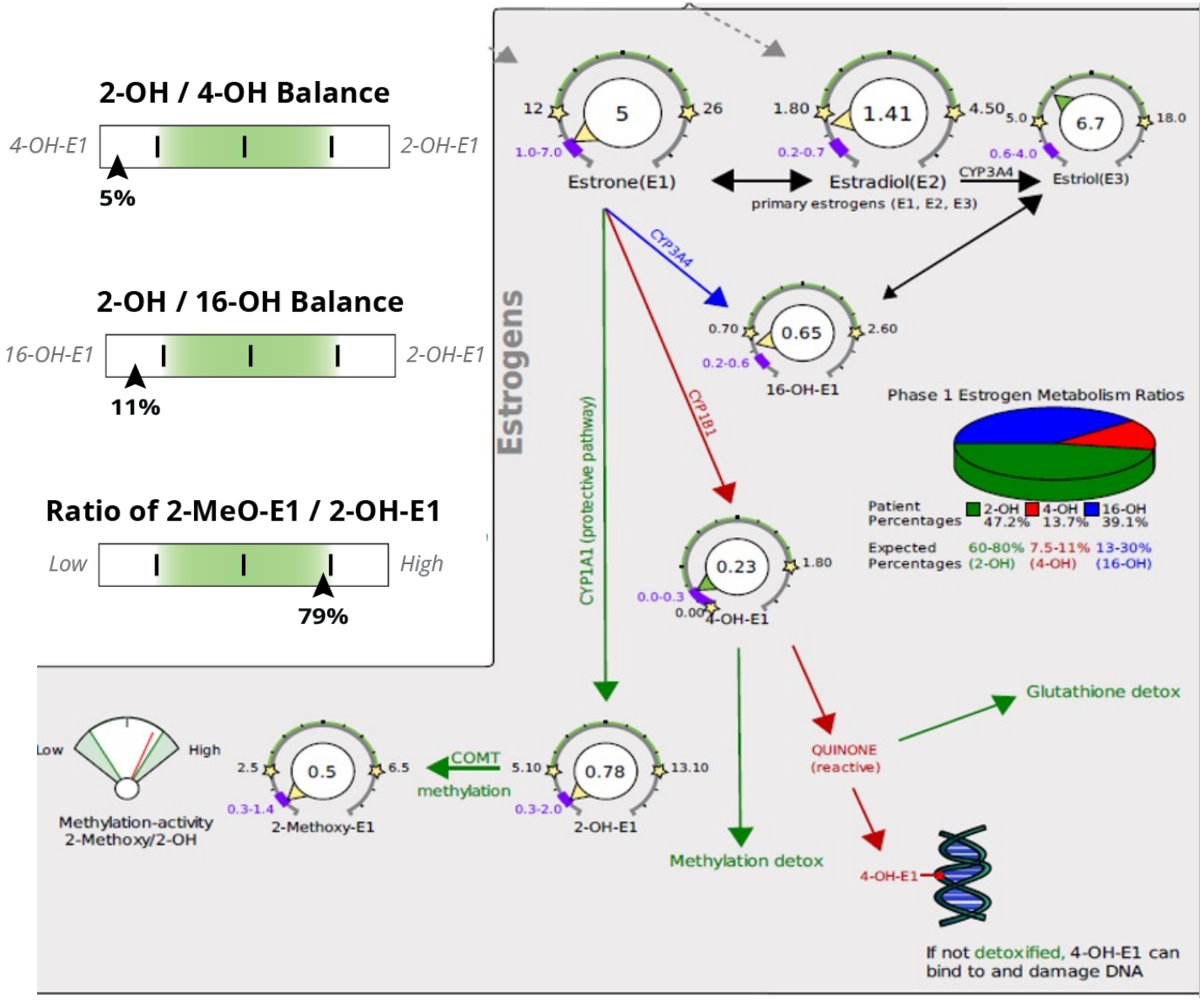
40-year-old female with irregular cycles.

High estrogen symptoms of fibrocystic breasts, mood swings, heavy menses.

Healthy Phase II methylation.

Consider Supporting the CYP1A1 pathway with sulforaphane, ground flax, or possibly DIM.

Consider supporting health 4OH with antioxidants like EGCG or NAC (or GSH)



2OH low

Low 2OH Case Points:

- In patients with irregular cycles, metabolite ratios are still accurate even if samples weren't collected at the right time.
- If 2OH is low, a careful review of parent estrogen levels compared to phase one metabolites can help.
- Separating out the 2:4 and 2:16 ratios can help further identify the balance of estrogen metabolism.

Treatment Considerations

CYP1A1

Our DUTCH Steroid pathways guide to the CYP-1A1 pathway.

CYP1A1 Increased	CYP1A1 Decreased
Cruciferous vegetables	High Sugar Diet
DIM/I3C	Moderate alcohol consumption
Caffeine	Resveratrol and pterostilbene
Soy	
Fish oil	
Rosemary extract	
Thyroxine	
Flaxseed	

CYP3A4

Our DUTCH Steroid pathways guide to the CYP-3A4 pathway.

Citations are at the end of this presentation

CYP3A4 Increases	CYP3A4 Decreases
St. John’s Wort	Grapefruit
Pesticides	Resveratrol
Caffeine	Rosemary
Smoking	Wild yam
PAHs	Peppermint oil
Moderate alcohol consumption	Azole antifungals
Obesity	

CYP1B1 Pathway

Our DUTCH Steroid pathways guide to the CYP-1B1 pathway.

Citations are at the end of this presentation.

CYP1B1 Increases (Bad)	CYP1B1 Decreases (Good)
Inflammation	Flavonoids
Smoking	Resveratrol
PAHs	

COMT Enzyme Activity

Our DUTCH Steroid pathways guide to the COMT pathway.

Citations are at the end of this presentation.

COMT Increase (Good)	COMT Decrease (Mostly bad)
SAMe	Estradiol
Magnesium	Phthalate esters
Choline	Rhodiola rosea
B6, B12, Folate	Quercetin
Betaine, Trimethylglycine	Catechin & epicatechins

DUTCH Interpretive Guide

The DUTCH Interpretive Guide is a useful tool for reviewing estrogen metabolism and contains valuable resources for treatment considerations.

Click the link in the chat to become a provider and gain access to the DUTCH Interpretive Guide.

Summary

Summary of Estrogen Metabolism

- Estrogen is a powerful hormone that has many health benefits.
- Estrogen also has a negative impact, increasing the risk of several common health issues.
- Detoxification of estrogen is an important step in regulating estrogen levels.
- Phase I Estrogen Detox has 3 pathways:
  - The 2OH pathway is protective
  - The 16OH pathway is proliferative and estrogenic
  - The 4OH pathway is genotoxic, oxidative
- Phase II Detox neutralizes phase I metabolites
  - The COMT enzyme methylates catechol estrogens 2OH and 4OH
  - Conjugation is also a neutralizing phase II pathway
- Measuring Estrogen Detox:
  - Follow the E1 pathway
  - Use both the levels of metabolites and the ratios of metabolites
  - Important ratios are:
    - 2:4 hydroxy
    - 2:16 hydroxy
    - 2Me/2OH (methylation rate)
  - Ratios can be seen in the pie chart and methylation gauge.

Thank You!

For questions, contact:

info@dutchtest.com

(503) 687-2050

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DUTCH Interpretive Guide

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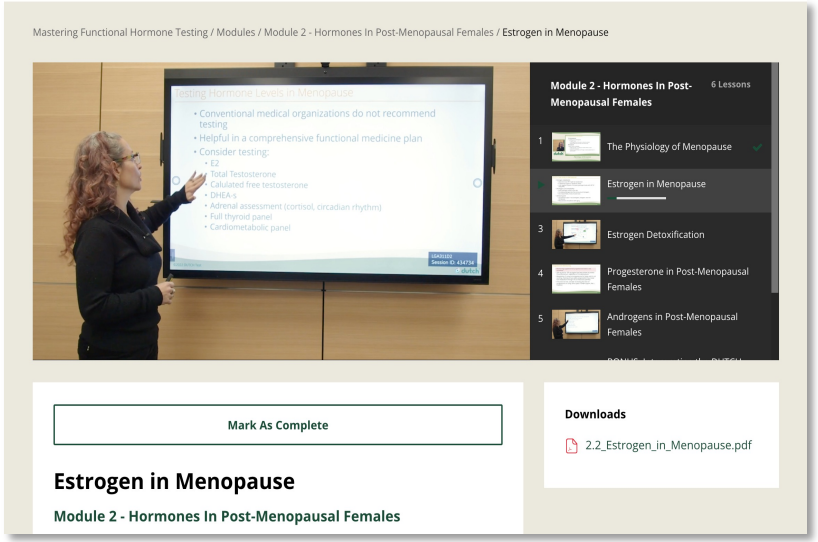

Group Mentorship Sessions

Click the Link Below to

Become a DUTCH Provider Today!

References:

Estrogen:

- https://www.breastcancer.org/facts-statistics
- https://www.breastcancer.org/risk/risk-factors
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- https://www.breastcancer.org/risk/risk-factors
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