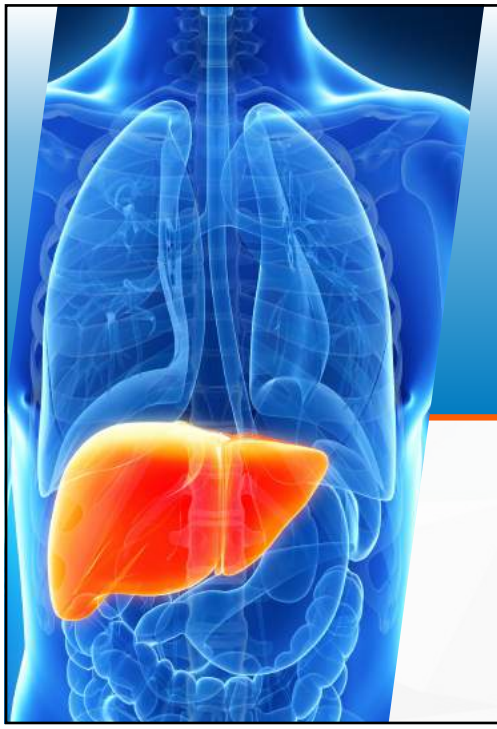


Get Out! Understanding Proper Estrogen Detoxification through Phases 1, 2, and 3 to Reduce Risk

Carrie Jones ND, MPH
Medical Director, Precision Analytical Inc.

1

Disclosure:
Dr. Carrie Jones is the Medical Director for Precision Analytical Inc.

2

Objectives

- Recognize ways in which Estrogen is cleared from the body
- Explain Phase 1, Phase 2 and Phase 3 Estrogen detox in the liver
- Identify the genetics involved in Phase 1, 2 and 3 liver detox
- Describe the gut microbiome's effect on Estrogen clearance
- Evaluate treatment options

3

Let's Set the Scene…

You make or take an estrogen.
You use the estrogen.
You are now done with that estrogen.

Where does the estrogen go?

4

First, before we cover anything

Nothing in your body is in a silo
Everything affects everything
Be aware of a domino effect

5

Which Estrogen Detox We'll Discuss:

1. Through the liver (and other extra hepatic tissues)

- Phase 1 detoxification via hydroxylation to 2, 4 and 16-OH
- Phase 2 detoxification via methylation and COMT

2. Through the GI Tract

- Phase 3 – looking at the estrobolome and its effect on glucuronidation

6

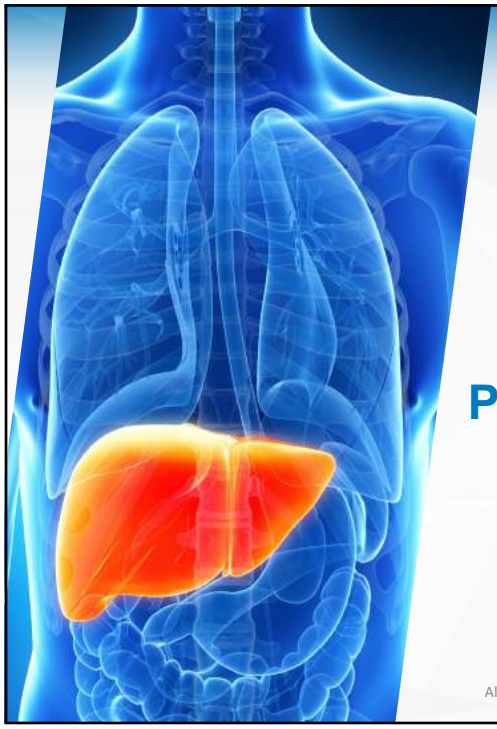
Why are we only discussing these?

There is specific marker testing available for hydroxylation, methylation and excretion of estrogen

7

However, there is snp testing available for all of the metabolism and conjugation pathways

8



Let's Start With:

Phase 1 and Phase 2 Detoxification

9

Why is Estrogen Metabolism Important?

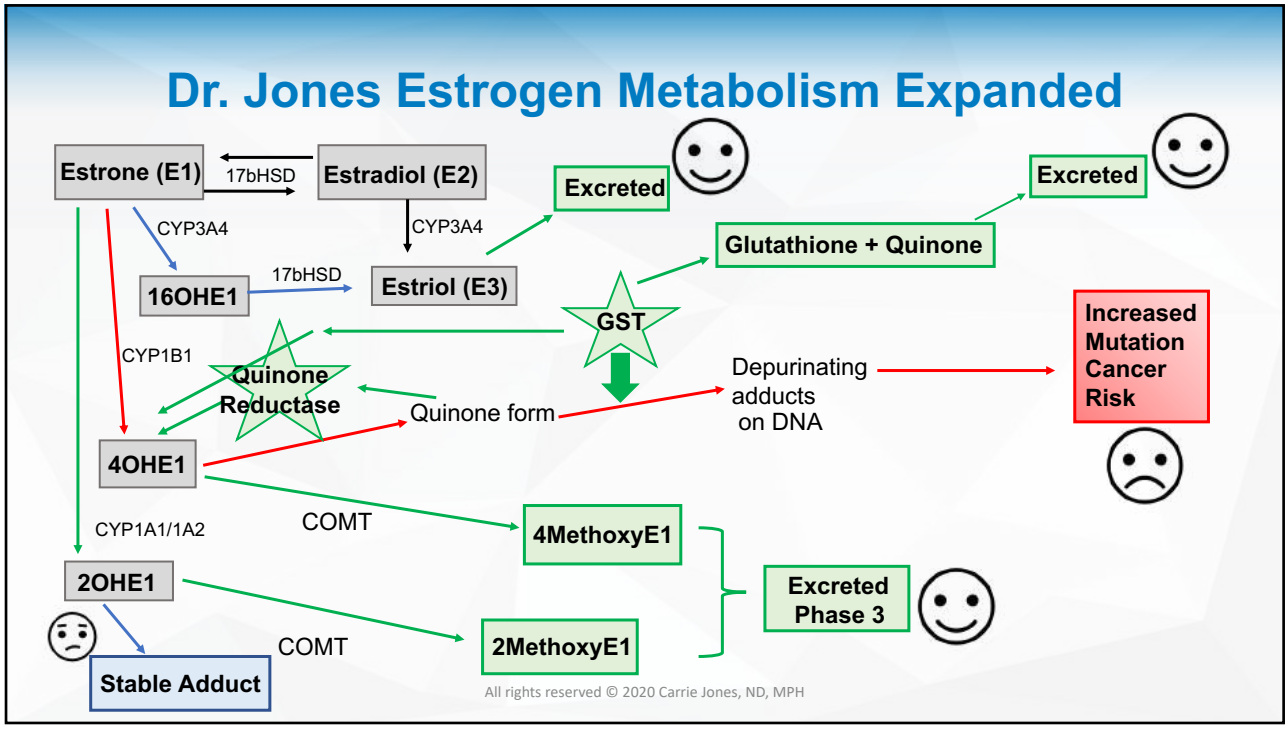
1

Understand
estrogen dominance
in men and women (luteal)

2

Understand/try to reduce cancer risk

10



11

Overall Phase 1 Goals:

1. Get to the Methoxy State
2. Induce GST and NQ01

And then excrete through the intestines

12

A Little About Each Metabolite

The phase-1 estrogen metabolites are considered free radicals in their semi-quinone or quinone states

2-OH (Phase-1) 😟 → **2-methoxy (Phase-2)** 🙂

- CYP activity primarily in the liver, but also extra-hepatically
- Considered the ***"less carcinogenic"*** metabolite
  - Binds to ERs with ***less affinity, not as tightly***
  - Forms ***'stable' adducts*** with DNA

- Methylated via COMT
- Considered ***anti-proliferative*** through microtuble disruption, apoptosis induction and angiogenesis inhibition
- Considered to have ***anti-aromatase*** activity
- Not thought to bind to the estrogen receptor (much)

13

A Little About Each Metabolite

The phase-1 estrogen metabolites are considered free radicals in their semi-quinone or quinone states

4-OH (Phase-1) ☹️

- CYP activity primarily in the breast, ovary, uterus, lung and kidney, minorly in the liver
- ***Much more carcinogenic*** because they form 'unstable' or 'depurinating' adducts
- Binds to the estrogen receptor with higher and tighter affinity than 2-OH
- E2 can increase CYP1B1 = higher 4-OH
  - Be aware with luteal phase estrogen dominance or excess estrogen replacement therapy

14

A Little About Each Metabolite

The phase-1 estrogen metabolites are considered free radicals in their semi-quinone or quinone states

Estriol = 16-OH-Estradiol

- Made primarily in the liver from ***16-hydroxylation of estradiol or dehydrogenation of 16-OH-E1***
- Very weak estrogen
- Binds ***very weakly to the estrogen receptor***
- Generally prefers ERb
- Antagonist at high levels at GPER (G-protein coupled estrogen receptor 1) = might help inhibit cancer cell growth

16-OH-Estrone (Phase-1)

- Metabolites of Estrone that can become Estriol (see above)
- Binds ***tightly/high affinity for the estrogen receptor***
- ***Associated with proliferation***
- Possibly upregulated by non-coffee caffeine (ie. soda)

15

Citations

- Girgert R, Emons G, Gründker C. Inhibition of GPR30 by estriol prevents growth stimulation of triple-negative breast cancer cells by 17β-estradiol. BMC cancer. 2014; 14:935. [pubmed]
- Hersey RM, Lloyd T, MacLusky NJ, Weisz J. The catechol estrogen, 2-hydroxyestradiol-17 alpha, is formed from estradiol-17 alpha by hypothalamic tissue in vitro and inhibits tyrosine hydroxylase. Endocrinology. 1982; 111(5):1734-6. [pubmed]
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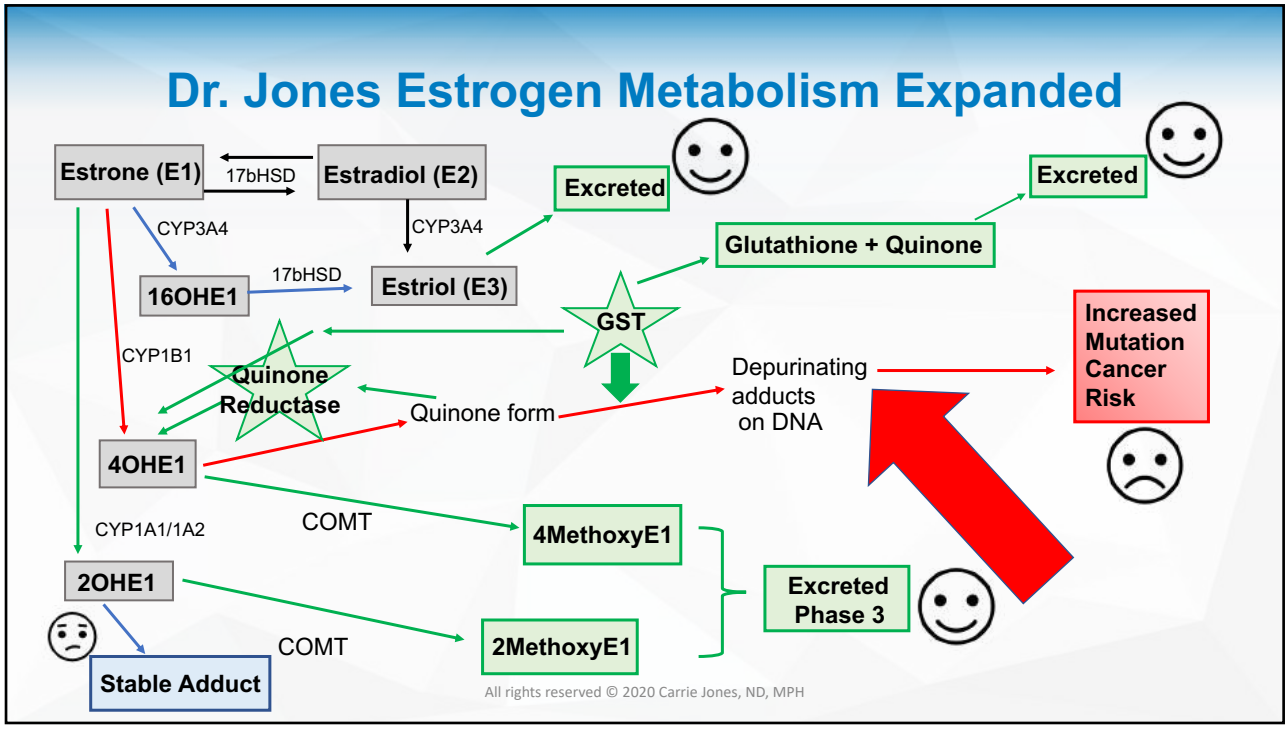
16


Dr. Jones Estrogen Metabolism Expanded
Estrone (E1)
17bHSD
Estradiol (E2)
Excreted
Excreted
CYP3A4
CYP3A4
Glutathione + Quinone
16OHE1
17bHSD
Estriol (E3)
GST
Increased Mutation Cancer Risk
CYP1B1
Quinone Reductase
Depurinating adducts on DNA
Quinone form
4OHE1
CYP1A1/1A2
COMT
4MethoxyE1
2OHE1
Excreted Phase 3
COMT
2MethoxyE1
Stable Adduct
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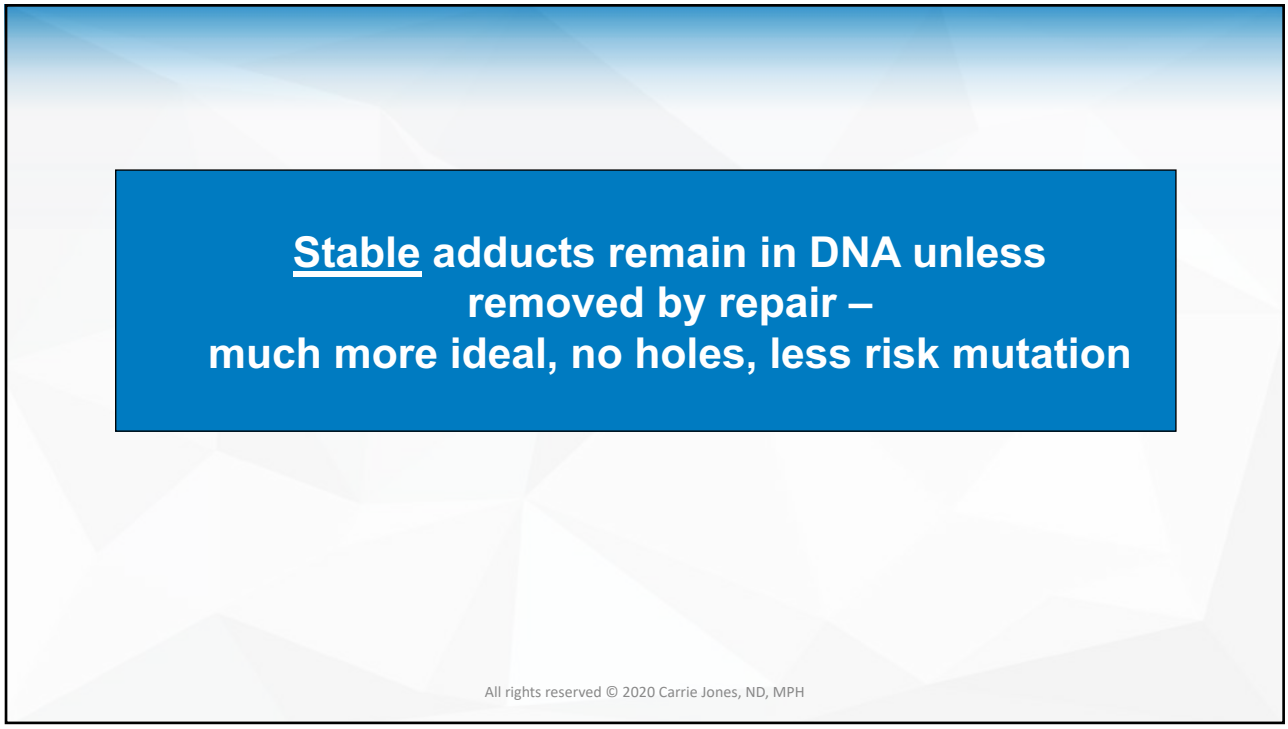
17


Stable adducts remain in DNA unless removed by repair –
much more ideal, no holes, less risk mutation
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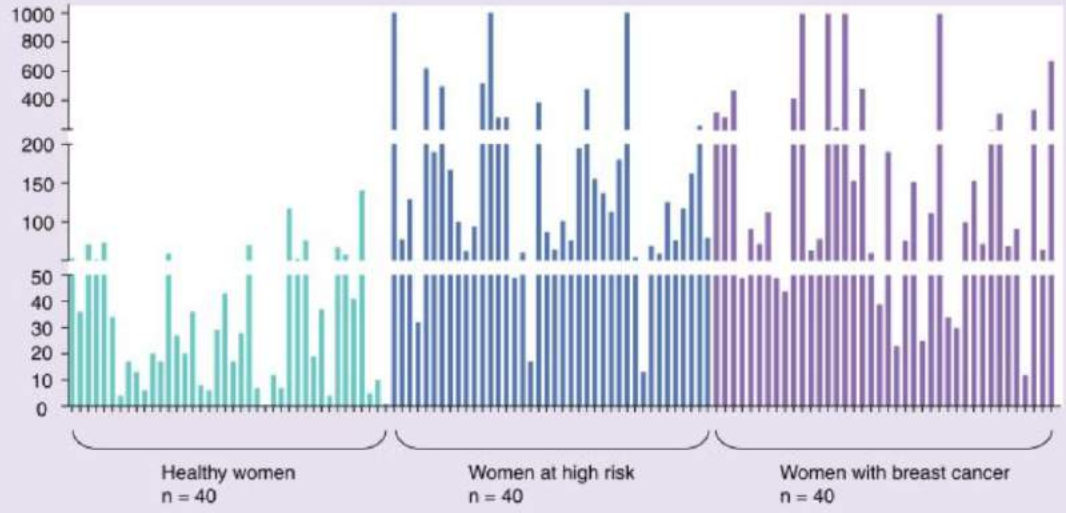
Depurinating (unstable) adducts break off from the DNA leaving a DNA with apurinic sites primarily at the N3 and N7 of Adenine and N7 of Guanine. Poor repair of these sites can cause mutations that lead to cancer.

19

Breast Cancer: Estrogen-DNA Adducts

Cavalieri E and Rogan E. Depurinating estrogen–DNA adducts in the etiology and prevention of breast and other human cancers. Future Oncol. 2015;6(1):75-91.



20

Citations:

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Estrogen Detoxification

Learn it as phase 1 → 2 → 3

Address it as phase 3 → 2 → 1

22

Detox Analogy: Picture a claw foot tub

Phase 1 is the water filling up the tub
- Is the right type of water filling up the tub?
- How fast or slow is it filling up?

Phase 2 is the drain
- How open or closed is the drain?
- Is it open wide enough?

Phase 3 is the sewer line out
- Is this dysfunctional causing backup?

23

How To Evaluate Phases 1 and 2

Through Urine Testing

24

Phase 1 Detoxification of Estrogen

25

Detox Analogy: Picture a claw foot tub

Phase 1 is the water filling up the tub
- Is the right type of water filling up the tub?
- How fast or slow is it filling up?

Phase 2 is the drain
- How open or closed is the drain?
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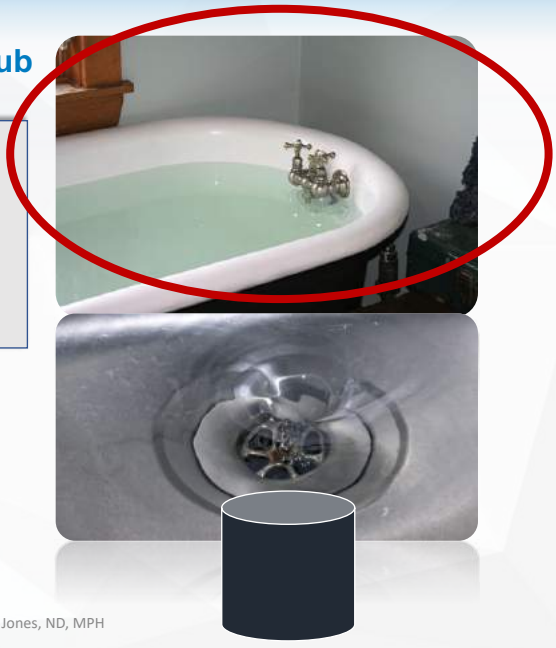
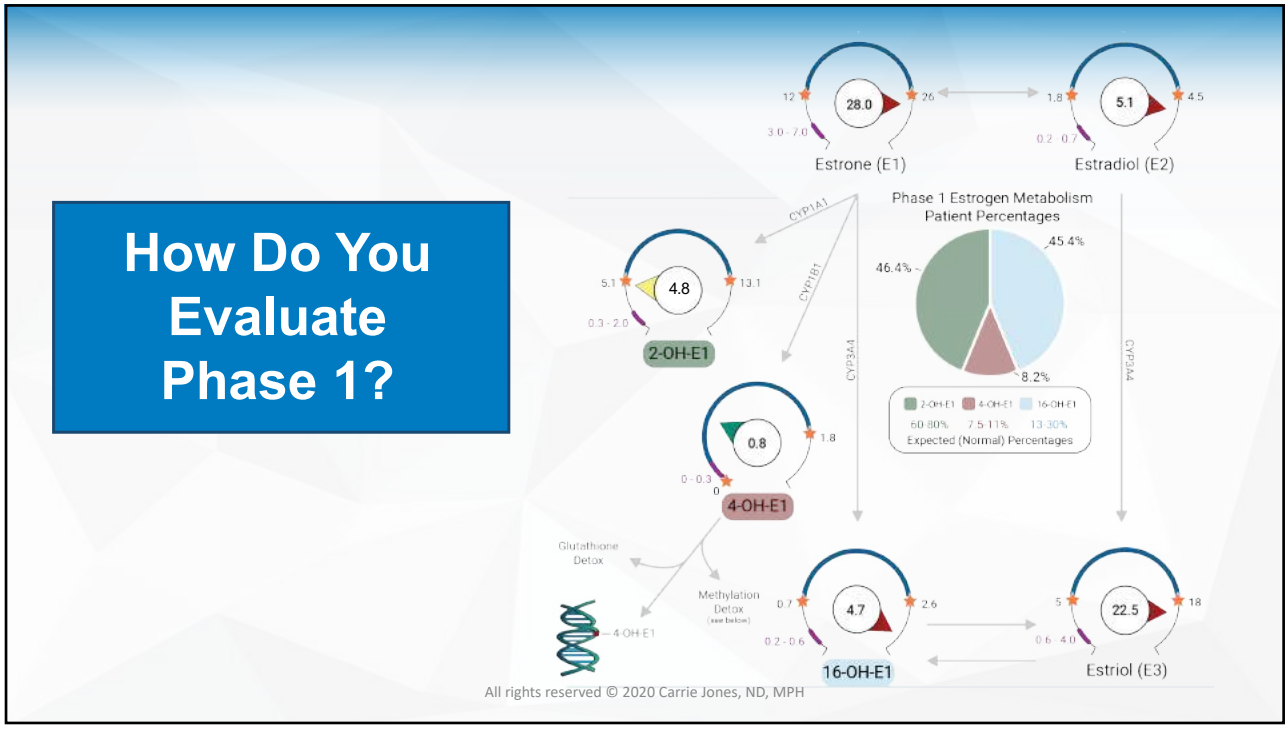
- Is this dysfunctional causing backup?



26


How Do You Evaluate Phase 1?
28.0
Estrone (E1)
5.1
Estradiol (E2)
Phase 1 Estrogen Metabolism
Patient Percentages
45.4%
46.4%
8.2%
Expected (Normal) Percentages
4.8
2-OH-E1
0.8
4-OH-E1
4.7
16-OH-E1
22.5
Estriol (E3)
CYP1A1
CYP1B1
CYP3A4
Glutathione Detox
Methylation Detox
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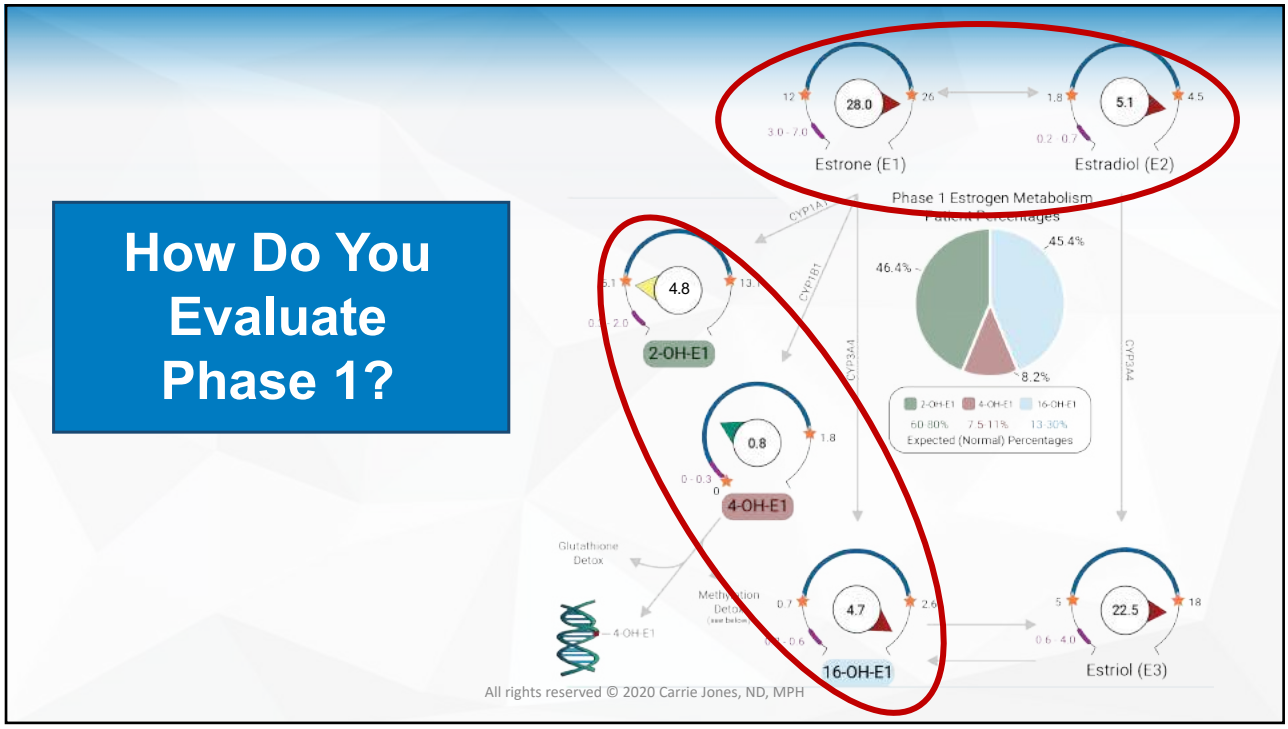
27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16-OH-E1
22.5
Estriol (E3)
CYP1A1
CYP1B1
CYP3A4
Glutathione Detox
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The CYP Family Preferences for Estrogen

- CYP1A1/1A2 → 2-OH
- CYP1B1 → 4-OH
- CYP3A4 → 16-OH

- Cribb AE. Role of Polymorphic Human Cytochrome P450 Enzymes in Estrone Oxidation Cancer Epidemiology Biomarkers & Prevention. 2006; 15(3):551-558.
- Sepkovic DW1, Bradlow HL. Estrogen hydroxylation--the good and the bad. Ann N Y Acad Sci. 2009 Feb;1155:57-67. doi: 10.1111/j.1749-6632.2008.03675.x.

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PSA #1:
These CYP450 enzymes do not ONLY work with estrogen

Please realize this when you want to directly upregulate or downregulate a pathway

30

PSA #2:
These CYP450 enzymes create free radicals

These can be highly mutagenic

Again, estrogen is not the only substrate they metabolize

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Be Thoughtful Before You Pull an Oprah

Image source: Oprah Winfrey Show/ You Tube

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What Affects Your CYP Family?

- **Iron** – CYP are heme dependent

- **Environmental Toxicants** – PAHs, PCBs, xenoestrogens (phthalates, BPA) (↑ CYP activity)
- **Smoking** (↑ CYP activity)
- **Charred Meats** (BBQ) (↑ CYP activity)
- **St. John's Wort** (↑CYP3A4)

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What Affects Your CYP Family?

- **Inflammatory Cytokines** (↓CYP1A and CYP3A4)
- **Grapefruit Juice** (↓CYP activity)
- **Ketoconazole** (↓CYP activity)
- **Verapamil** (↓CYP3A4)
- **Propranolol** (↓CYP1A2)
- **Berberine** (↓CYP3A4)
- **Resveratrol** (↓CYP1B1)
- **Alcohol Consumption** (↓2-OH and ↑16-OH)
- **Artemisia** (↓CYP1A)
- **Tagamet (Cimetidine)** (↓CYP1A)

Not an extensive list

34

Citations: CYP Family

- Bailey DG, Dresser GK. Interactions Between Grapefruit Juice and Cardiovascular Drugs American Journal of Cardiovascular Drugs. 2004; 4(5):281-297.
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- Larson B and Banks P. Automated 384-Well Cell-Based Cytochrome P450 Inhibition Assays Using Cryopreserved Human Hepatocytes in Suspension. 2012; Retrieved on February 12, 2020 from https://www.biotek.com/resources/application-notes/automated-384-well-cell-based-cytochrome-p450-inhibition-assays-using-cryopreserved-human-hepatocytes-in-suspension/
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- Whitten DL, Myers SP, Hawrelak JA, Wohlmuth H. The effect of St John's wort extracts on CYP3A: a systematic review of prospective clinical trials Br J Clin Pharmacol. 2006; 62(5):512-526.
- Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation Pharmacology & Therapeutics. 2013; 138(1):103-141.

35

Phase 1:
Estrogen Optimization Strategies:
DIM/I3C
Di-Indolylmethane or Indole-3-Carbinol

36

I3C

Phase 1 Metabolism Primarily

- **I3C** needs stomach acid to then convert into 15+ compounds, of which the most predominant is DIM
  - 10-60% of I3C converts to DIM

37

DIM

Phase 1 Metabolism Primarily

- An acid catalyzed dimer of I3C
  - DIM increases in brassica family if the food is chopped or mildly heated
  - Out of the body within about 24hrs
- DIM increases the conversion of E1/E2 into 2-OH-E1 via CYP1A

How?

- Binds to aryl hydrocarbon receptor (AhR) = induces CYP1A mostly selectively
  - CYP1B1 activation is much less compared to CYP1A1 activation (invitro/animal)
- It also induces Nrf2 (invitro/animal studies)
- **Dosage in research:** 100-300mg/day

38

DIM

Phase 1 Metabolism Primarily

- An acid catalyzed dimer of I3C
  - DIM increases in brassica family if the food is chopped or mildly heated
  - Out
- DIM inc

How?

- Binds t y selectively
  - CYP imal)
- It also induces Nrf2 (invitro/animal studies)
- **Dosage in research:** 100-300mg/day

I3C and DIM *may* be challenging in the UK
Look to other support instead

39

Can't I Just Eat the Brassica Family Foods?

"On average, 100 g of cruciferous vegetables contains up to 30 mg of glucobrassicin, which is estimated to convert to approximately 2 mg of DIM. However, the variation in DIM content between different cruciferous vegetables is considerable, with differences ranging from 5- to 8-fold. To achieve a biologically relevant exposure, it is suggested that intake would need to be upwards of 600 g/d and sustained for several years to achieve an anticancer benefit. An intake this high is difficult to attain or maintain through diet alone."

Thomson CA, Ho E, Strom MB. Chemopreventive properties of 3,3'-diindolylmethane in breast cancer: evidence from experimental and human studies. *Nutrition Reviews*. 2016;74(7):432-443. doi:10.1093/nutrit/nuw010.

40

41

Possible DIM Concerns?

- DIM can lower estrone/estradiol levels in circulation by increasing phase 1 metabolism = careful in those with low E levels
- 2014 breast cancer cells study found DIM ***in the absence of estradiol***, low levels of DIM (10uM) ***activated*** ERa and ***increased*** breast cancer proliferation.
  - At high levels (50uM) DIM was antiproliferative
  - This has not been done in human studies

Marques M, Laflamme L, Benassou I, Cissokho C, Guillemette B, Gaudreau L. Low levels of 3,3′-diindolylmethane activate estrogen receptor α and induce proliferation of breast cancer cells in the absence of estradiol BMC Cancer. 2014; 14(1).

42

Citations: I3C/DIM

- Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC cancer. 2009; 9:187. [pubmed]
- Ernst IMA, Schuemann C, Wagner AE, Rimbach G. 3,3′-Diindolylmethane but not indole-3-carbinol activates Nrf2 and induces Nrf2 target gene expression in cultured murine fibroblasts Free Radical Research. 2011; 45(8):941-949.
- Szaefer H, Licznerska B, Krajka-Kuźniak V, Bartoszek A, Baer-Dubowska W. Modulation of CYP1A1, CYP1A2 and CYP1B1 Expression by Cabbage Juices and Indoles in Human Breast Cell Lines Nutrition and Cancer. 2012; 64(6):879-888.
- Thomson CA, Ho E, Strom MB. Chemopreventive properties of 3,3'-diindolylmethane in breast cancer: evidence from experimental and human studies. Nutrition reviews. 2016; 74(7):432-43.]
- Wu T, Khor TO, Su Z, et al. Epigenetic Modifications of Nrf2 by 3,3′-diindolylmethane In Vitro in TRAMP C1 Cell Line and In Vivo TRAMP Prostate Tumors AAPS J. 2013; 15(3):864-874.

43

Please Keep in Mind…

1. 2-OH is not healthy per se – just ‘less carcinogenic’
2. 2-OH can still form adducts
3. The ***goal*** is to make 2-methoxy quickly and upregulate NQ01 and GST
4. Remember treatment is 3 → 2→1 = don’t put everyone on DIM
5. CYP1A1/1A2/1B1 is not only for estrogen
   - Be aware if you modulate them.

Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC cancer. 2009; 9:187. [pubmed]

44

Phase 1:
Estrogen Optimization Strategies:
Quercetin

45

Quercetin

- Potent CYP1B1 inhibitor
- Less effective inhibitor of CYP1A1 and 1A2
- High bioavailability of flavonoids (20%) when taken orally
- Also commonly used for reducing histamine and pro-inflammatory cytokines
- Typical dose: 250-2000mg/day

46

Citations: Quercetin

- Androutsopoulos VP, Papakyriakou A, Vourloumis D, Spandidos DA. Comparative CYP1A1 and CYP1B1 substrate and inhibitor profile of dietary flavonoids Bioorganic & Medicinal Chemistry. 2011; 19(9):2842-2849.
- Martinez-Perez C, Ward C, Cook G, et al. Novel flavonoids as anti-cancer agents: mechanisms of action and promise for their potential application in breast cancer . 2014; 42(4):1017-1023.
- Mlcek J, Jurikova T, Skrovankova S, Sochor J. Quercetin and Its Anti-Allergic Immune Response. Molecules (Basel, Switzerland). 2016; 21(5):623. [pubmed]
- Yang T, Feng Y, Chen L, Vaziri ND, Zhao Y. Dietary natural flavonoids treating cancer by targeting aryl hydrocarbon receptor Critical Reviews in Toxicology. 2019; 49(5):445-460.

47

Phase 1:
Estrogen Optimization Strategies:
Coffee

48

Coffee and Estrogen

Multivariate-adjusted[1] geometric means of estrogen metabolism measures by category of coffee intake

	≤6 cups/week	1 cup/day	2–3 cups/day	4+ cups/day	
N	293	73	181	40	
Individual and grouped EM (pmol/mg creatinine)					
Total EM	195.5	197.0	207.2	222.7	0.08
Parent estrogens	40.5	41.8	41.2	44.4	0.37
Estrone	26.1	28.0	27.6	28.5	0.29
Estradiol	13.5	12.5	13.3	14.7	0.51
Catechols	**61.2**	**62.0**	**69.4**	**88.5**	**0.002**
2-catechols	**51.9**	**54.4**	**59.7**	**79.0**	**0.001**
2-Hydroxyestrone	**45.7**	**48.4**	**52.6**	**69.6**	**0.001**
2-Hydroxyestradiol	**5.4**	**5.3**	**6.1**	**8.4**	**0.001**
4-catechols					
4-Hydroxyestrone	6.1	5.5	6.1	7.1	0.61
Methylated catechols	11.2	10.2	10.4	10.7	0.49

Phase 1 only

Sisti JS, Hankinson SE, Caporaso NE, et al. Caffeine, coffee and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2015;24(8):1174-1183. doi:10.1158/1055-9965.EPI-15-0246.

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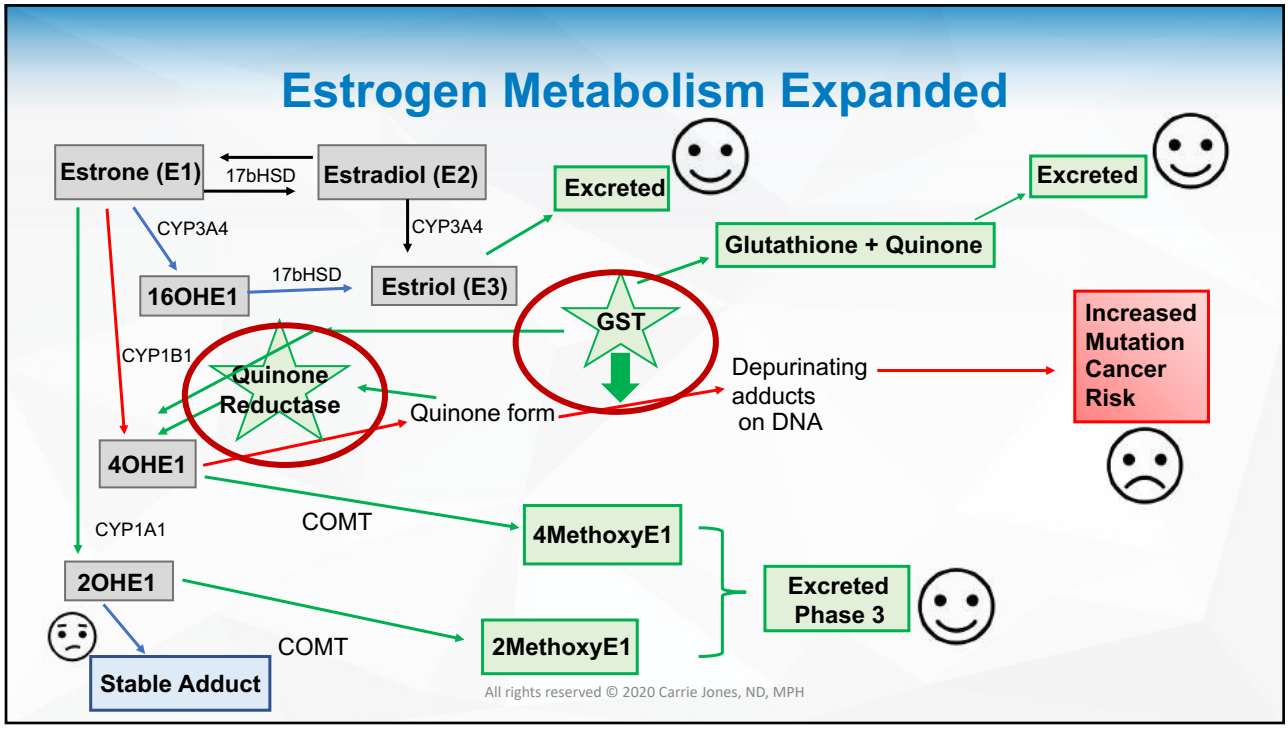
What Else Helps Reduce Risk and Symptoms?

50



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NQ01 = NAD(P)H: Quinone Reductase
GST = Glutathione S-Transferase

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Lower Risk/Reduce Symptoms: NAC

- Phase 1(ish) and 2 Metabolism
- NAC = N-Acetyl-Cysteine
- Prevent damage to DNA by inhibiting formation of catechol quinones and/or reacting with them
  - Can also reduce the E2-3,4semiquinone back into 4-OH-E2
  - NAC inhibited adduct formation by 60% or more by reacting with quinone itself in in vitro studies
- Hydrolysis of NAC in the liver and gut → cysteine to help make **glutathione**

Zahid M, Gaikwad M, Rogan E and Cavalieri E. Inhibition of Depurinating Estrogen DNA Adduct Formation by Natural Compounds. Chem. Res. Toxicol. 2007,20,1947–1953.

53

NAC for the Win

"Towards this goal, in a preliminary in vitro study, we have tested several antioxidants, NAcCys, GSH, cysteine (Cys), melatonin, resveratrol and reduced lipoic acid. **NAcCys was found to be one of the best inhibitors** of formation of depurinating adducts in reaction mixtures containing E2-3,4-Q or enzyme activated 4-OHE2 and DNA"

Zahid M, Gaikwad M, Rogan E and Cavalieri E. Inhibition of Depurinating Estrogen DNA Adduct Formation by Natural Compounds. Chem. Res. Toxicol. 2007,20,1947–1953.

54

NAC Supplementation Lowers DNA Adducts

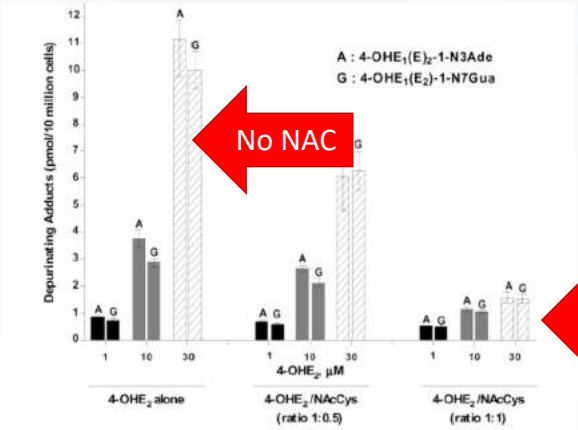


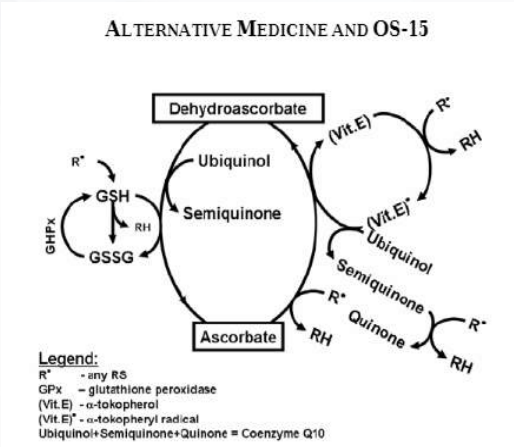
Figure 4.
Effects of NAcCys on the formation of estrogen-DNA adducts in MCF-10F cells treated with 4-OHE_2.

55

Can't I Just Give Straight Glutathione?

- Yes! Maybe make sure you can recycle it first
- **Co-factors:** Selenium, FAD, vitamin C, vitamin E, CoQ10, ALA, magnesium, zinc, B6, folate and B12

- **Did you know:**
  - Vitamin C recycles Vitamin E and ALA
  - CoQ10 recycles Vitamin C and Vitamin E
  - Vitamin E recycles Vitamin C and CoQ10
  - ALA recycles Vitamin C and CoQ10



https://www.researchgate.net/publication/311486840_ALTERNATIVE_MEDICINE_AND_CONTROL_OVER_THE_OXIDATIVE_STRESS

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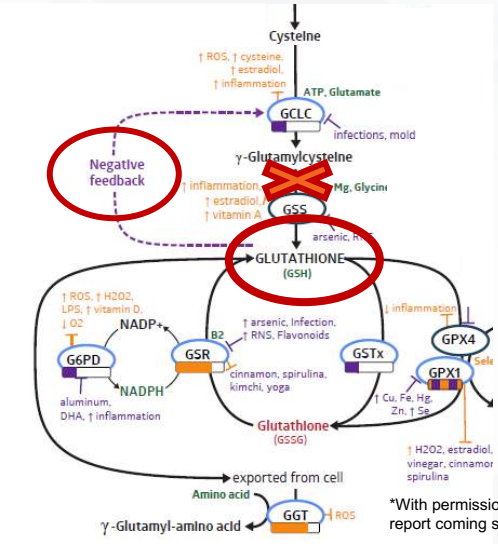
Is there a glutathione feedback loop?



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What Else Helps Reduce Risk and Symptoms?

Sulforaphane

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Lower Risk/Reduce Symptoms: Suforaphane/Glucoraphanin

- Compound in isothiocyanate group from cruciferous veggies
- Made when the enzyme Myrosinase is released from chopping/chewing and combines with Glucoraphanin = **Sulforaphane**
- Broccoli sprouts have highest concentration as do cauliflower and mustard sprouts

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Lower Risk/Reduce Symptoms: Sulforaphane/Glucoraphanin

- **Supplementation:**
  - Must be standardized to include glucoraphanin and myrosinase that will yield a sulforaphane amount
  - Some supplement companies provide only glucoraphanin and rely on the microbiome to convert it to sulforaphane
    - This yields wildly varying amounts of sulforaphane due to differing microbiomes
  - "Sulforaphane glucosinolate" is ONLY glucoraphanin – don't be mislead
  - Epithiospecifier protein (ESP) in crucifers inhibit myosinase to a degree
    - Some supplement companies do not screen for or deactivate ESP in the supplement

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Lower Risk/Reduce Symptoms: Sulforaphane/Glucoraphanin

- **Broccoli sprouts:**
  - Must be chopped, cut or chewed to create sulforaphane
  - Studies suggest 0.5-1 teaspoon (2-5 grams) of powder is necessary
  - Cooking, microwaving, steaming, boiling, has all been shown to reduce glucosinolates within minutes
  - Open air storage and storing in a plastic bag has been shown to reduce glucosinolates within 3-7 days

Houghton CA, Fassett RG, Coombes JS. Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality? Oxidative Medicine and Cellular Longevity. 2016; 2016:1-17.

61

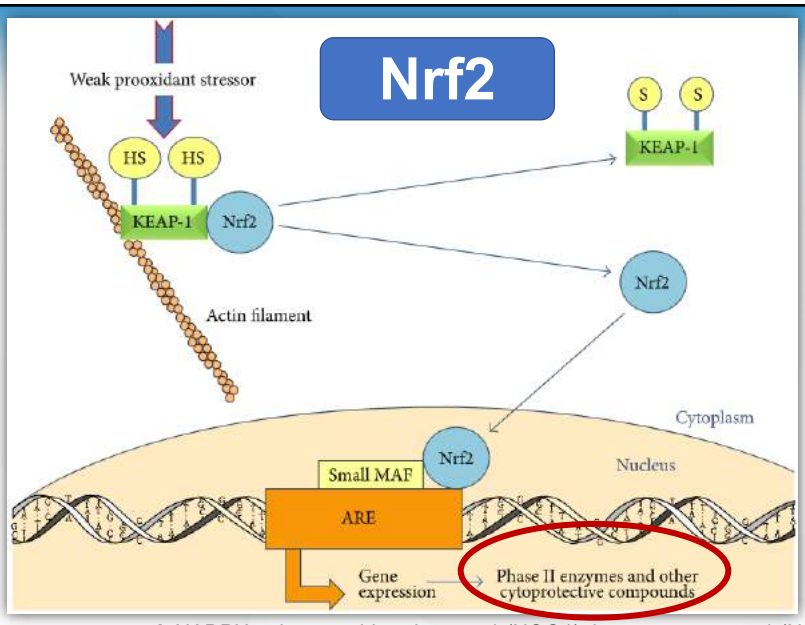

Image: Houghton CA, Fassett RG, Coombes JS. Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality? Oxidative Medicine and Cellular Longevity. 2016; 2016:1-17.

Enzymes overexpressed: NADPH:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and transketolase (TKT)

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What Else Helps Reduce Risk and Symptoms?

Resveratrol
(*trans*-3,4',5-trihydroxystilbene)

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The Thing About Resveratrol

- In grapes, peanuts, wine: Cabernet Franc wine supposed to be best wine to raise Quinone reductase (but it's still alcohol).
  - Wine averages 8.2 uM (per liter)
- Classified as a phytoestrogen – agonist or antagonist at ER depending on concentration, competition and expression of ER in the cells

Stervbo U., Vang O., Bonnesen C. A review of the content of the putative chemopreventive phytoalexin resveratrol in red wine. Food Chem. 2007;101:449–457. doi: 10.1016/j.foodchem.2006.01.047

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The Thing About Resveratrol: Good

- It's an antioxidant – reduces reactive oxygen species (ROS)
- It's an AHR *antagonist*
  - Inhibits CYP1B1 specifically
  - **"Its inhibitory ability for CYP1B1 is over 50-fold greater than against CYP1A1 and 500-fold higher than for CYP1A2"** (Li, et al, 2017).
  - Induces Nrf2 = increasing glutathione peroxidase, glutathione S-transferase and Quinone Reductase
  - This reduces E2-3,4-semiquinone back into 4-OH-E2 instead
- It's anti-tumor (via p53 tumor suppressor and other mechanisms)
- All from invitro and animal studies

Li F, Zhu W, Gonzalez FJ. Potential role of CYP1B1 in the development and treatment of metabolic diseases Pharmacology & Therapeutics. 2017; 178:18-30.

65

The Thing About Resveratrol: Bad

- It is not very bioavailable and undergoes rapid phase II metabolism in the liver/microbiome, unfortunately, so much study is done invitro and or in animals as levels aren't likely high enough in humans to make much impact with supplementation or diet
  - 5 grams of resveratrol supplementation produces a transient serum peak of 2.4uM in vivo (moles per liter of blood)
- The in vivo studies are typically days to 3 months – long enough?
- But, it's possible, the metabolites of resveratrol are potent and helpful too and are being studied – there are 21-ish metabolites

Stervbo U., Vang O., Bonnesen C. A review of the content of the putative chemopreventive phytoalexin resveratrol in red wine. Food Chem. 2007;101:449–457. doi: 10.1016/j.foodchem.2006.01.047

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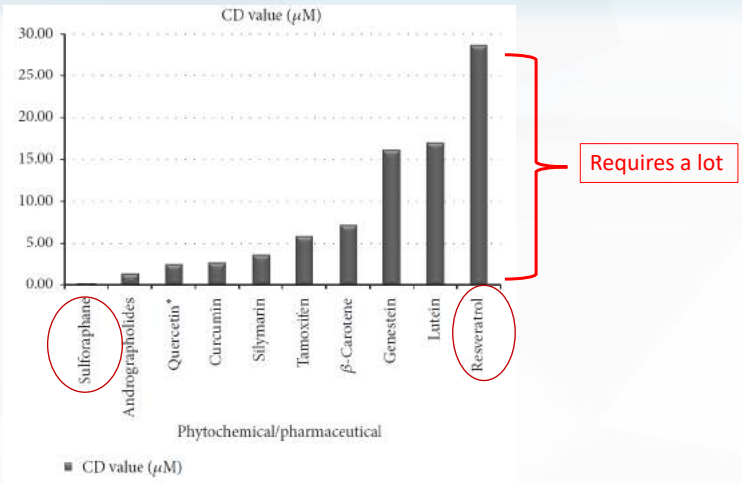
The Thing About Resveratrol: Humans

- In 15 subjects, 40mg of *trans* resveratrol was 8.8-fold higher compared to dry powder non-trans resveratrol
- The ½ life of *trans* resveratrol is 2-5 hours with repeat dosing
- Micronized resveratrol (less than 5mm) for 14 days produced higher plasma concentration over non-micronized

Ramírez-Garza SL, Laveriano-Santos EP, Marhuenda-Muñoz M, et al. Health Effects of Resveratrol: Results from Human Intervention Trials. *Nutrients*. 2018;10(12):1892. Published 2018 Dec 3. doi:10.3390/nu10121892

67

Sulforaphane/Resveratrol & Nrf2



Review Article

Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality?

Christine A. Houghton, Robert G. Fassett, and Jeff S. Coombes

FIGURE 2: CD values of popular phytochemicals used as supplements and a commonly prescribed pharmaceutical. CD values refer to the concentration of a compound required to double the activity of the Phase II detoxification enzyme, quinone reductase [83, 87–89, 91].

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Maybe We've Been Looking at it Wrong

- At the Integrative Healthcare Symposium 2020 in NYC, Dr. Jeffrey Bland and Dr. Tom Guilliams suggested that poor bio-available compounds (like resveratrol and curcumin) might actually be working through microbiome signaling
- Therefore, serum levels will not be high, but there is still a positive systemic effect

- Bird JK, Raederstorff D, Weber P, Steinert RE. Cardiovascular and Antiobesity Effects of Resveratrol Mediated through the Gut Microbiota. Adv Nutr 2017;8(6):839-49.
- Chen ML, Yi L, Zhang Y, Zhou X, Ran L, Yang J, Zhu JD, Zhang QY, Mi MT. Resveratrol attenuates trimethylamine-N-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. mBio 2016;7(2). doi: 10.1128/mBio.02210-15
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- Ling KH, Wan ML, El-Nezami H, Wang M. Protective Capacity of Resveratrol, a Natural Polyphenolic Compound, against Deoxynivalenol-Induced Intestinal Barrier Dysfunction and Bacterial Translocation. Chem Res Toxicol 2016;29(5):823-33.

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Citations:NAC/GST and Sulforaphane

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2. Cavalieri EL, Rogan EG, Zahid M. Critical depurinating DNA adducts: Estrogen adducts in the etiology and prevention of cancer and dopamine adducts in the etiology and prevention of Parkinson's disease Int. J. Cancer. 2017; 141(6):1078-1090.
3. Examine.com. Calcium d-Glucarate. Retrieved on July 7, 2017 from https://examine.com/supplements/calcium-d-glucarate/
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Citations: NQ01

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Citations: Resveratrol

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2. Ferraz da Costa D, Fialho E, Silva J. Cancer Chemoprevention by Resveratrol: The p53 Tumor Suppressor Protein as a Promising Molecular Target Molecules. 2017; 22(6):1014. Published online.
3. Li F, Zhu W, Gonzalez FJ. Potential role of CYP1B1 in the development and treatment of metabolic diseases Pharmacology & Therapeutics. 2017; 178:18-30.
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Phase 2
Detoxification of Estrogen

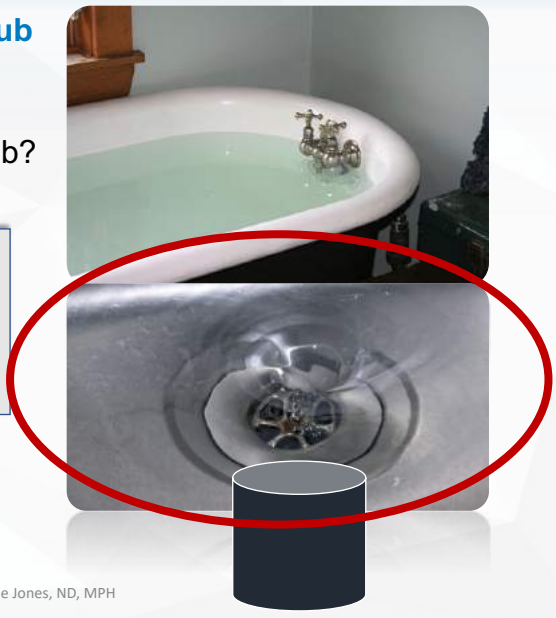
73

Detox Analogy: Picture a claw foot tub

Phase 1 is the water filling up the tub
- Is the right type of water filling up the tub?
- How fast or slow is it filling up?

Phase 2 is the drain
- How open or closed is the drain?
- Is it open wide enough?

Phase 3 is the sewer line out
- Is this dysfunctional causing backup?



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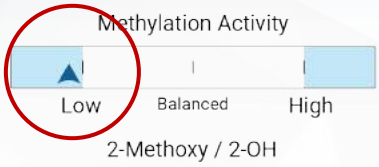
Estrogen Dominant Symptoms (Male or Female) Phase-2 – Why?



Gene & Variation	rsID	Alleles	Result
COMT V158M	rs4680	AA	+/+
COMT H62H	rs4633	TT	+/+
COMT P199P	rs769224	GG	-/-
MTHFR C677T	rs1801133	GG	-/-
MTHFR 03 P39P	rs2066470	GG	-/-
MTHFR A1298C	rs1801131	TT	-/-

3-4-fold decreased COMT activity

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What is COMT? Catechol-O-methyltransferase

- An enzyme encoded by the COMT gene
  - Conjugates **catechol estrogens**
    - 2-hydroxy Estrogen
    - 4-hydroxy Estrogen
  - Conjugates **catecholaminergic neurotransmitters**
    - Dopamine
    - Norepinephrine
    - Epinephrine

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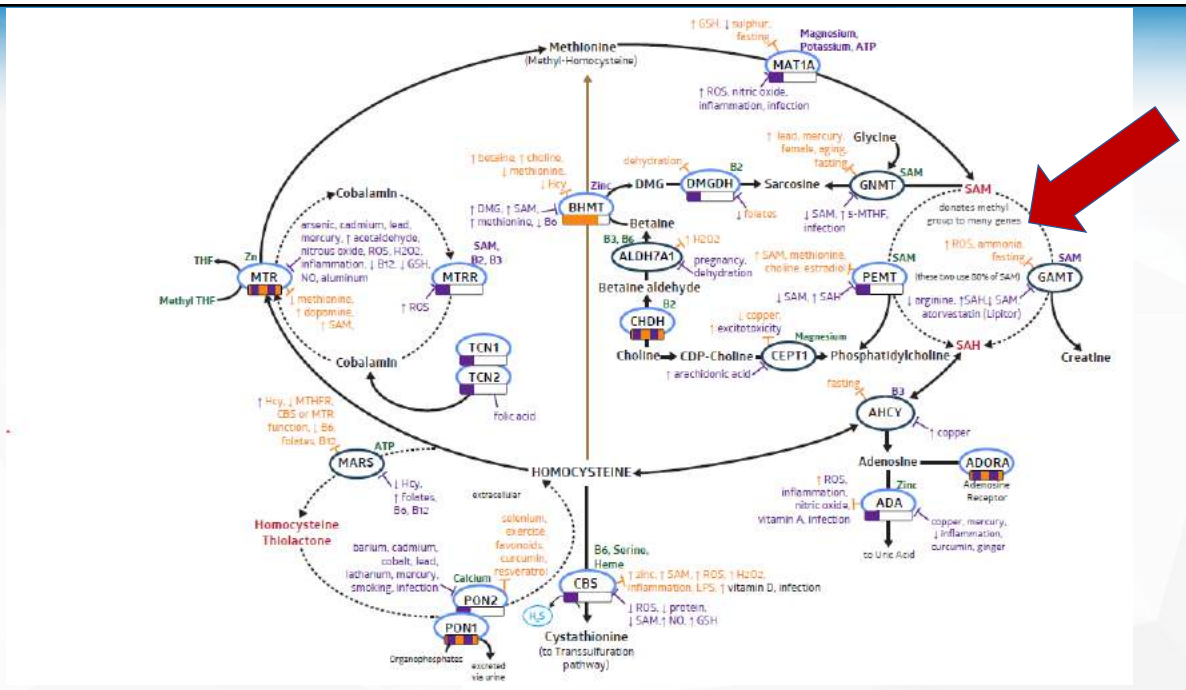
How Does COMT Work?

- The very short version:
- Works by using a methyl group donated by S-adenosyl methionine (SAM – the main co-factor) = “neutralizes” it
- Magnesium is used for proper binding of the catechol and SAM
- Zinc might be as effective as magnesium

Tsao D, Diatchenko L, Dokholyan NV. Structural mechanism of S-adenosyl methionine binding to catechol O-methyltransferase. PloS one. 2011; 6(8):e24287. [pubmed]

77



With permission from Dr. Ben Lynch from the new StrateGene report coming soon

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Free Radic Biol Med. 2007 December 1; 43(11): 1534–1540.

Inhibition of catechol-*O*-methyltransferase increases estrogen-DNA adduct formation

Abstract

The association found between breast cancer development and prolonged exposure to estrogens suggests that this hormone is of etiologic importance in the causation of the disease. Studies on estrogen metabolism, formation of DNA adducts, carcinogenicity, cell transformation and mutagenicity have led to the hypothesis that reaction of certain estrogen metabolites, predominantly catechol estrogen-3,4-quinones, with DNA forms depurinating adducts [4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua]. These adducts cause mutations leading to the initiation of breast cancer. Catechol-*O*-methyltransferase (COMT) is considered an important enzyme that protects cells from the genotoxicity and cytotoxicity of catechol estrogens, by preventing their conversion to quinones. The goal of the present study was to investigate the effect of COMT inhibition on the formation of depurinating estrogen-DNA adducts. Immortalized human breast epithelial MCF-10F cells were treated with 4-OHE_2 (0.2 or 0.5 μM) for 24 h at 120, 168, 216, and 264 h post-plating or one time at 1–30 μM 4-OHE_2 with or without the presence of COMT inhibitor (Ro41-0960). The culture media were collected at each point, extracted by solid-phase extraction and analyzed by HPLC connected with a multichannel electrochemical detector. The results demonstrate that MCF-10F cells oxidize 4-OHE_2 to $E_1(E_2)$-3,4-Q, which react with DNA to form the depurinating N3Ade and N7Gua adducts. The COMT inhibitor Ro41-0960 blocked the methoxylation of catechol estrogens, with concomitant 3–4 fold increases in the levels of the depurinating adducts. Thus, low activity of COMT leads to higher levels of depurinating estrogen-DNA adducts that can induce mutations and initiate cancer.

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What Slows Down/Affects COMT?

- COMT mutation (+/+)
- Being estrogen dominant (even elevated estrogen catechols)
- Gut infections – phenols from gut bacteria
- Certain Phenols from foods/supplements
  - Green tea (EGCG), quercetin
- High levels of SAH (blocks methyltransferases)
  - Consider Sam/SAH ratio
- Bisphenol and PCB exposure
- Serotonin can competitively block SAM receptors in COMT
- COMT inhibitors (medications)
- Early life adversity (ie. victim of abuse, robbery, rape, childhood trauma…etc.)
- **Really anything that affects the methionine cycle**

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Phase-2 Detox Requires COMT and Methyl Donors

- SAMe
  - main methyl donator
- Magnesium
  - Needed for proper binding
- Zinc
  - Might also be effective for binding, also part of the methionine cycle
- Tri-methyl-glycine
- Betaine
- Methionine
- Choline
- Methylated B vitamins

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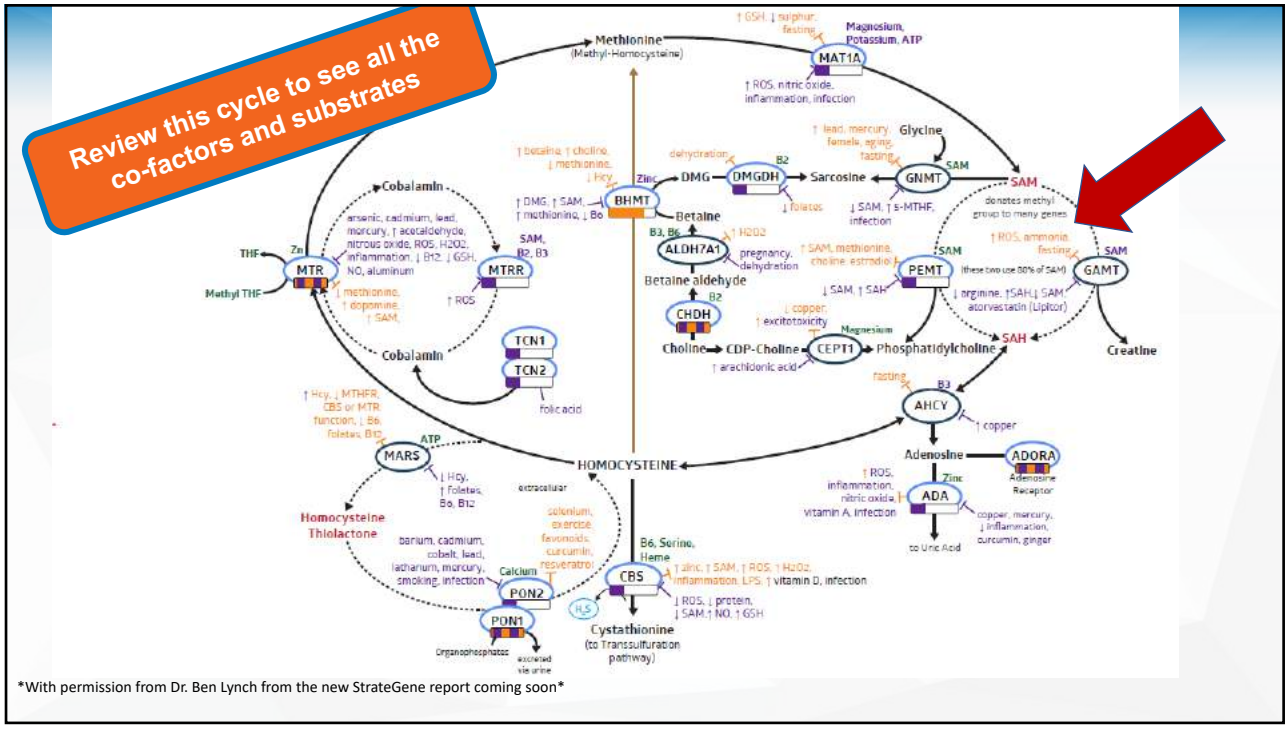

With permission from Dr. Ben Lynch from the new StrateGene report coming soon

82

Citations:

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83

Why is Phase 2 Detox Important?

It makes the phase 1 metabolites
(that are harmful reactive oxygen species – ROS)
water soluble to be excreted

84

Phase 2.5 and 3 Detoxification of Estrogen

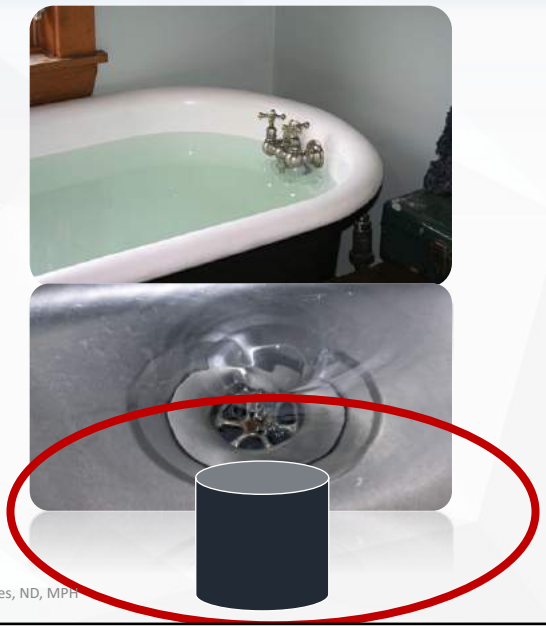
85

Detox Analogy: Picture a claw foot tub

Phase 1 is the water filling up the tub
- Is the right type of water filling up the tub?
- How fast or slow is it filling up?

Phase 2 is the drain
- How open or closed is the drain?
- Is it open wide enough?

Phase 3 is the sewer line out
- Is this dysfunctional causing backup?



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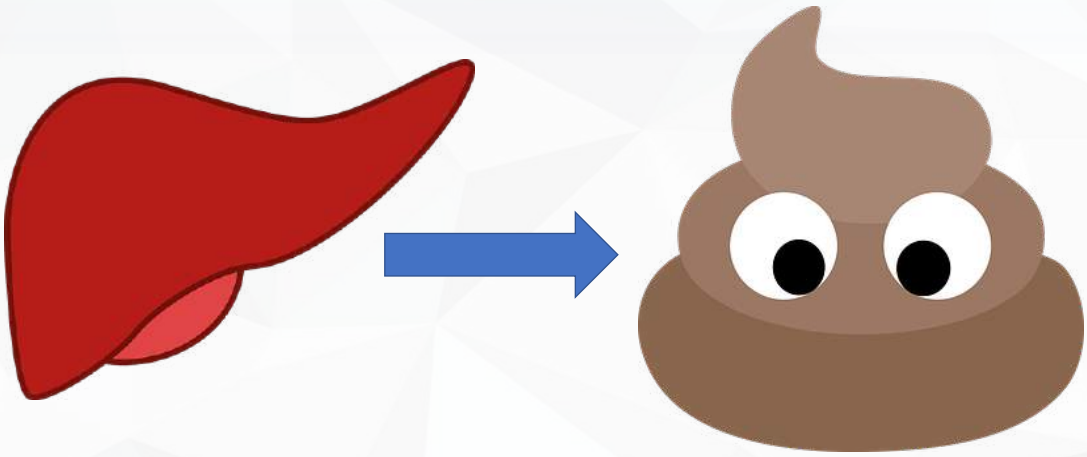
How Do You Evaluate Phases 2.5 and 3?



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What the Heck is 2.5 Detox?

- Once estrogen metabolites are water-soluble, they have to get **OUT** of the cell and excreted.
- Cell membranes are lipophilic
- The phase 2 estrogen metabolites are hydrophilic
- This is a problem

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Enter: The Transporter

- Phase 3 transporters known as: ATP-binding cassette (ABC)
  - Main one is P-glycoprotein (multidrug resistant protein1) & Breast Cancer Resistance Protein (BCRP)
- They move many things out of the cell:
  - Drugs/medications
  - Chemicals/toxicants
  - Hormones…etc.
- Once out of the cell into the interstitial fluid – they move into the kidney/urine and bile/intestines
  - There are lots of transporters at the BBB too

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What Slows Down Your Transporters? ☹

- **Inflammation**
  - Any and all inflammation (length of time might matter)
- Oxidative stress
- Being overburdened with toxicity
- Specific medications like azithromycin, clarithromycin, proton pump inhibitors (ie. Omeprazole)
- **Flavonoids like: Quercetin, rutin,** theaflavine, epicatechin 3-gallate, naringin, apigenin, hesperidin, proanthocyanidin, tangeritin, luteolin, baicalein, and daidzein (all cell studies)

90

What Supports Your Transporters?

- **Doing everything to reduce inflammation, oxidative stress and toxicity**
- **Nrf2 activators:**
  - Sulforaphane
  - Curcumin/Turmeric
  - Berberine
  - Resveratrol
  - Catechins in chocolate and green tea
  - DIM
  - Not quercetin
- **Cell membrane support**:
  - Choline
  - Healthy fats and oils (support bile)

91

Citations: Phase 2.5

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Bile Support:

- **Phosphatidyl Choline (Lecithin)** - improves bile formation and transport, reduces gallstone formation
- **Betaine** – improves bile formation and decreases fatty accumulation in the liver
- **TUDCA -** Tauroursodeoxycholic acid
- **Glycine and taurine** – use to make salts
- **Silymarin (Milk thistle)** – improves bile formation, hepatocyte protection
- **Ox bile** – to help replace bile especially if gallbladder is removed (note: most studies reported are from 1900-1950)
- **Bitters such as Gentian** – increase bile formation
- **Dandelion root (Taraxacum off)** – improve lipid metabolism and bile formation
- **Greater Celandine** – may support bile production and protect hepatocytes

93

Citations: Bile

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What About Estrogen and the Gut Microbiome (Phase 3)?

95

The Estrobolome!

- "…the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens"
- "By modulating the enterohepatic circulation of estrogens, the estrobolome affects both the excretion and circulation of estrogens. In turn, the composition of the estrobolome can be shaped by factors such as antibiotics, other drugs, and diet that modulate its functional activity."

Plottel SC, Blaser MJ. Microbiome and malignancy. *Cell Host Microbe.* 2011;10(4):324-335

96

- "Studies of injected radioactively labeled estradiol, estrone, and estriol in women indicate that approximately 65% of injected estradiol, 48% of injected estrone, and 23% of injected estriol are recovered in bile. As approximately 10% to 15% of injected radiolabeled estradiol, estrone, and estriol are found in conjugated form in feces, a biologically significant proportion of estrogens are reabsorbed in the circulation. Hepatically conjugated estrogens excreted in the bile can be deconjugated by bacterial species with ß-glucuronidase activity in the gut, leading to their reabsorption into the circulation."

Maryann Kwa, Claudia S. Plottel, Martin J. Blaser, Sylvia Adams; The Intestinal Microbiome and Estrogen Receptor–Positive Female Breast Cancer. J Natl Cancer Inst 2016; 108 (8): djw029. doi: 10.1093/jnci/djw029

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"GUSs"

Gut Bacterial β-D-Glucuronidases

98

What Does Beta-Glucuronidase Do?

- It is an enzyme in colonocytes
- It 'deconjugates' glucuronidated substances
  - Removes glucuronic acid from the estrogen!
- The GUSB gene (encodes for b-Glucuronidase) is well represented in phyla Bacteroidetes and Firmicutes

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What Does Beta-Glucuronidase Do to Estrogen?

- **Analogy:**
  - The body puts estrogen in a box and ties it up with a bow to be excreted in the stool (glucuronidation).
  - Beta-glucuronidase unties the bow and opens the lid allowing estrogen to escape (reactivates it) and **get reabsorbed**

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Is beta-glucuronidase all bad?

No, it does help break down large carbohydrates and things like flavonoids plus assists with bilirubin reabsorption

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Maturitas. 2017 Sep;103:45-53. doi: 10.1016/j.maturitas.2017.06.025. Epub 2017 Jun 23.

Estrogen-gut microbiome axis: Physiological and clinical implications.

Baker JM[1], Al-Nakkash L[2], Herbst-Kralovetz MM[3].

Author information

Abstract

The gut microbiota regulates estrogens through secretion of β-glucuronidase, an enzyme that deconjugates estrogens into their active forms.
The alteration in circulating estrogens may contribute to the development of conditions discussed herein: obesity, metabolic syndrome, cancer, endometrial hyperplasia, endometriosis, polycystic ovary syndrome, fertility, cardiovascular disease (CVD) and cognitive function.

detailed. Modulation of the microbiome composition subsequently impacts the metabolic profile, and vice versa, and has been shown to alleviate many of the estrogen-modulated disease states. Last, we highlight promising research interventions in the field, such as dietary therapeutics, and discuss areas that provide exciting unexplored topics of study.

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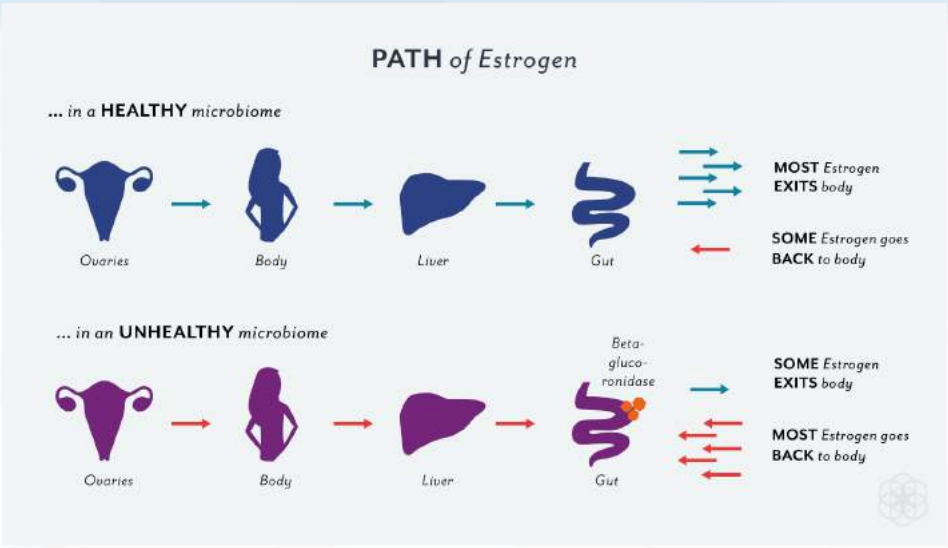

https://medium.com/clued-in/how-your-gut-affects-your-hormones-bc8f67509721

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What can you do to create a healthy estrobolome?

Test – Don't Guess!

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1. Focus on Diet (The Basics)

J Transl Med. 2017; 15: 73.
Published online 2017 Apr 8. doi: 10.1186/s12967-017-1175-y
PMCID: PMC5385025
PMID: 28388917

Influence of diet on the gut microbiome and implications for human health

Rasnik K. Singh,[1] Hsin-Wen Chang,[2] Di Yan,[2] Kristina M. Lee,[2] Derya Ucmak,[2] Kirsten Wong,[2] Michael Abrouk,[3] Benjamin Farahnik,[4] Mio Nakamura,[2] Tian Hao Zhu,[5] Tina Bhutani,[2] and Wilson Liao[2]*

Author information ▶ Article notes ▶ Copyright and License information ▶ Disclaimer

"…it is now understood that diet plays a significant role in shaping the microbiome, with experiments showing that dietary alterations *can induce large, temporary microbial shifts within 24 h*."

microbiome, with experiments showing that dietary alterations can induce large, temporary microbial shifts within 24 h. Given this association, there may be significant therapeutic utility in altering microbial composition through diet. This review systematically evaluates current data regarding the effects of several common dietary components on intestinal microbiota. We show that consumption of particular types of

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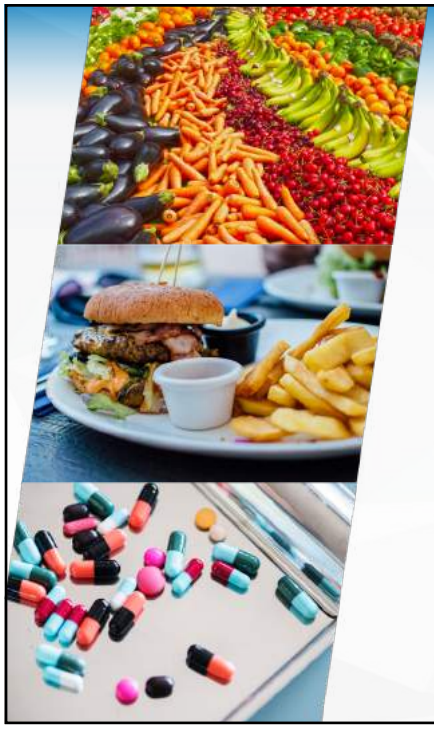

What Increases Beta-Glucuronidase?

- **Low consumption of:**
  - Fruits and vegetables
  - Fiber
- **High consumption of:**
  - Sugar
  - Processed foods
  - 'Standard American Diet'
  - Alcohol
  - Toxicants
  - Antibiotics

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2. Prebiotics and Resistant Starch

Table 5

Effects of non-digestible carbohydrates on gut microbiota

	Bacterial abundance	Gene richness	*Lactobacilli*	*Bifidobacteria*	*Clostridia*	*Enterococcus*	*Roseburia*	*Eubacteria*	*Ruminococcus*	References
Fiber/prebiotics	↑	↑	↑	↑	↓	↑↓				[30, 64–66, 69–76]
Resistant starch	↑	↑	↑	↑			↑	↑	↑	[3, 30, 67–69, 72–74]

J Transl Med. 2017; 15: 73. Published online 2017 Apr 8. doi: 10.1186/s12967-017-1175-y

Examples:

- ❑ **Artichoke (Inulin), dandelion greens, jicama, chicory root…etc**
- ❑ **Partially hydrolyzed guar gum**
- ❑ **Pectin**

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Citations:

- Ghaffarzadegan T, Marungruang N, Fåk F, Nyman M. Molecular Properties of Guar Gum and Pectin Modify Cecal Bile Acids, Microbiota, and Plasma Lipopolysaccharide-Binding Protein in Rats. PloS one. 2016; 11(6):e0157427. [pubmed]
- Niv E, Halak A, Tiommny E, et al. Randomized clinical study: Partially hydrolyzed guar gum (PHGG) versus placebo in the treatment of patients with irritable bowel syndrome. Nutrition & metabolism. 2016; 13:10. [pubmed]
- Vandeputte D, Falony G, Vieira-Silva S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut. 2017; 66(11):1968-1974. [pubmed]
- Yang X, Darko KO, Huang Y, et al. Resistant Starch Regulates Gut Microbiota: Structure, Biochemistry and Cell Signalling. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2017; 42(1):306-318. [pubmed]

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2. Probiotics and Fermented Foods

Table 6

Effects of probiotics on gut microbiota

*Studies evaluated looked at fermented foods and taking probiotics

	Bacterial abundance	*Bifidobacteria*	*Lactobacilli*	*Streptococcus*	Total aerobes/anaerobes	Total coliforms	*Helicobacter pylori*	*Escherichia coli*	References
Probiotics	↑	↑	↑	↑	↑	↓	↓	↓	[84–98]

J Transl Med. 2017; 15: 73. Published online 2017 Apr 8. doi: 10.1186/s12967-017-1175-y

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3. Raw, Unpeeled, Organic Carrots

- **Raw carrots** - fiber is mostly insoluble cellulose
- There is pectin in the carrot cell wall
- Insoluble fiber is beneficial to the gut:
  - Promote GI regularity
  - Increases stool bulk
  - Increases short chain fatty acid production (protective)
  - Decreases beta glucuronidase and sulfatase
  - Decreases the opportunity for hormones/toxins to be reabsorbed
- **Other foods higher in insoluble fiber:** wheat bran, apples/pears (with skin on), rice, blackberries, green peas, amaranth, almonds, pistachios and avocado

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Citations: Carrots/Insoluble Fiber

- Borisenkov MF, Karmanov AP, Kocheva LS, et al. Adsorption of -glucuronidase and estrogens on pectin/lignin hydrogel particles International Journal of Polymeric Materials and Polymeric Biomaterials. 2015; 65(9):433-441.
- Landete JM. Plant and mammalian lignans: A review of source, intake, metabolism, intestinal bacteria and health. Food Research International. 2012;46(1):410-424.
- Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. Nutrients. 2010; 2(12):1266-89. [pubmed]
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- McRorie JW, McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber Journal of the Academy of Nutrition and Dietetics. 2017; 117(2):251-264.
- Rose DP, Goldman M, Connolly JM, Strong LE. High-fiber diet reduces serum estrogen concentrations in premenopausal women . 1991; 54(3):520-525.
- Sharma KD, Karki S, Thakur NS, Attri S. Chemical composition, functional properties and processing of carrot—a review J Food Sci Technol. 2011; 49(1):22-32.
- Soliman GA. Dietary Fiber, Atherosclerosis, and Cardiovascular Disease Nutrients. 2019; 11(5):1155 (published online).

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4. Reduce Antibiotics and Toxicant Exposure

"…use of antibiotics reduces the capacity of intestinal microflora to metabolize phytochemicals into compounds that may protect against cancer. However, antibiotic use also disrupts the intestinal microfloral metabolism of estrogens."


February 18, 2004

Antibiotic Use in Relation to the Risk of Breast Cancer

Christine M. Velicer, PhD; Susan R. Heckbert, MD, PhD; Johanna W. Lampe, PhD, RD; et al

JAMA. 2004;291(7):827-835. doi:10.1001/jama.291.7.827

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Toxicant Exposure

Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model

Trends in Endocrinology & Metabolism
Volume 28, Issue 8, August 2017, Pages 612-625

Review

Gut Microbiota, Endocrine-Disrupting Chemicals, and the Diabetes Epidemic

Chemical transformation of xenobiotics by the human gut microbiota

Nitzan Koppel, Vayu Maini Rekdal, Emily P. Balskus

Science 23 Jun 2017:
Vol. 356, Issue 6344, eaag2770
DOI: 10.1126/science.aag2770

Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta.

Overview of air pollution and endocrine disorders

Philippa D Darbre

Siciliano, Steven. "Human colon microbiota transform polycyclic aromatic hydrocarbons to estrogenic metabolites." *The Free Library* 01 January 2005. 27 March 2018 <https://www.thefreelibrary.com/Human colon microbiota transform polycyclic aromatic hydrocarbons to...-a0136511528>.

[Environmental Chemical Exposure and Its Effects on Infants' Reproductive Hormones].

[Article in Japanese]
Araki A, Itoh S, Miyashita C, Minatoya M, Kishi R.

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5. Eliminate or Reduce Alcohol: SIBO

Moderate alcohol consumption is associated with small intestinal bacterial overgrowth, study finds

Date: November 28, 2011

Source: American College of Gastroenterology

Summary: Just one drink per day for women -- two for men -- could lead to small intestinal bacterial overgrowth and subsequently cause gastrointestinal symptoms like bloating, gas, abdominal pain, constipation and diarrhea, according to the results of a new study.

<www.sciencedaily.com/releases/2011/10/111031114949.htm>

114

5. Eliminate or Reduce Alcohol: Increasing Estrogen

- Key TJ, Appleby PN, Reeves GK, et al. for the Endogenous Hormones and Breast Cancer Collaborative Group. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. Br J Cancer. 105(5):709-22, 2011.
- Hamajima N, Hirose K, Tajima K, et al. for the Collaborative Group on Hormonal Factors in Breast Cancer. Alcohol, tobacco and breast cancer—collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer. 87(11):1234-45, 2002
- Erol A, Ho AM, Winham SJ, Karpyak VM. Sex hormones in alcohol consumption: a systematic review of evidence. 2017. Addict Biol. Dec 27. doi: 10.1111/adb.12589. [Epub ahead of print]
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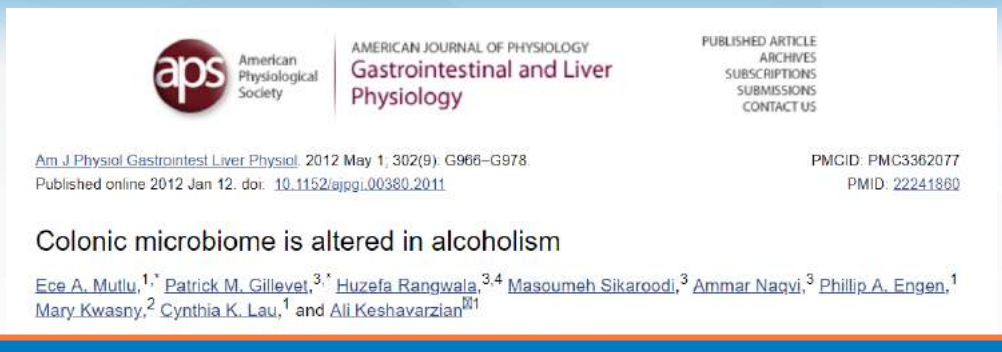

"A subgroup of alcoholics have an altered colonic microbiome (dysbiosis). The alcoholics with dysbiosis had lower median abundances of Bacteroidetes and higher ones of Proteobacteria. The observed alterations appear to correlate with **high levels of serum endotoxin** in a subset of the samples. Network topology analysis indicated that alcohol use is correlated with decreased connectivity of the microbial network, **and this alteration is seen even after an extended period of sobriety."**

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Please don't try to negotiate your alcohol choices with me… you know the studies

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6. Supplement: Calcium-d-Glucarate

- **CDG =** b-glucuronidase inhibitor

- **Also known as**:
  - Calcium glucarate, Saccharic acid, Calcium-D-Saccharate, Glucaric acid
- **Do not confuse it with:**
  - Calcium, Glutamine or Saccharin

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6. Supplement: Calcium-d-Glucarate

- **MOA:**
  - Keeps the glucuronide group on the hydrophobic molecule for excretion (urine/GI)
  - It does NOT lower b-glucuronidase levels in the gut
- **Dosing is VERY high:**
  - 1500-3000mg/day at a minimum (in animal studies, they were on much higher doses based on mg/kg of body weight)
  - However, it appears that every bit helps (ie. 500mg is still helpful)
- **Foods high in CDG**
  - (but not anywhere near therapeutic doses): oranges, carrots, apples, grapefruit, and cruciferous vegetables

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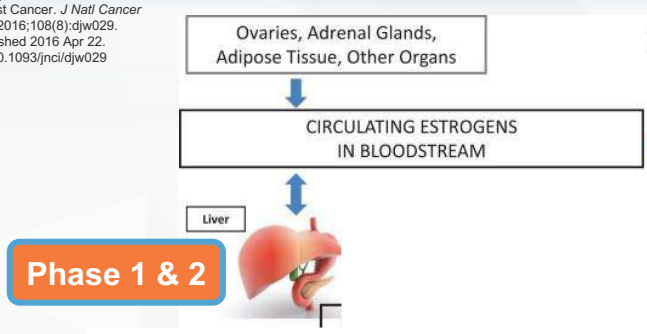
Let's Summarize: Visual

Kwa M, Plottel CS, Blaser MJ, Adams S. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J Natl Cancer Inst.* 2016;108(8):djw029. Published 2016 Apr 22. doi:10.1093/jnci/djw029



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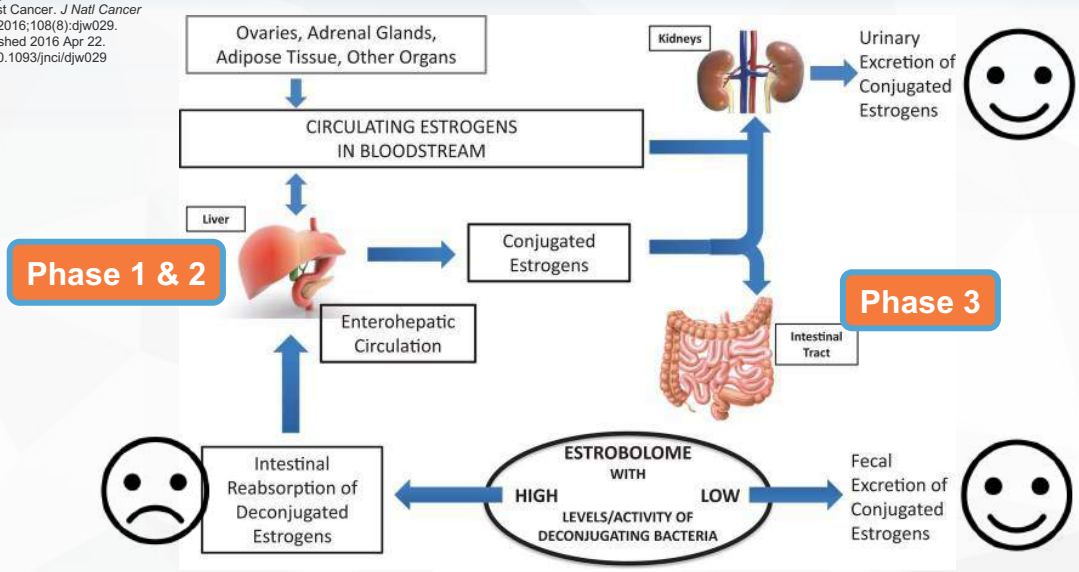

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Let's Summarize:

1. Learn it as Phase 1 → 2 → 3 but **<u>address it as 3 → 2→ 1</u>** ★
2. The creation of unstable adducts is a real problem
3. If you don't test estrogen metabolism via urine, you won't know where the problem lies.
4. Look to the COMT, NQ01, and GST gene to help with proper excretion
5. Don't forget about your transporters! *(Eat dark chocolate)*
6. Unhealthy bile and an unbalanced microbiome will raise hormone levels
7. Do stool and/or a SIBO test for further evaluation
8. You are what you eat/absorb and what your microbiome does with you so eat for the liver and your microbiome
9. If you choose to drink alcohol, drink Cabernet Franc (apparently)

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…and that concludes our talk

Thank you for listening.

Lecture questions?
info@dutchtest.com

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