Estrogen Balance, Clearance, and Health

Anu Desai, PhD

Lyra Heller, MA

Metagenics Institute

Learning Objectives

1. Explore clinical implications of disrupted estrogen metabolism and how modulation may help reduce risk in hormone-related health concerns
2. Understand how estrogen is produced, utilized, and cleared by the body
3. Explain how key nutritional influences can support healthy estrogen biotransformation and clearance

Halbreich U et al. *Psychoneuroendocrinology*. 2003;28 Suppl 3:1–23.

Estrogen Can Influence a Women's Health for 50+ Years

The aging reproductive system

	Reproductive Years			Menopausal Transition			Postmenopause
Average age	1st period: 9-15	16-30	31-42	Early transition: 40s	Late transition: late 40s–early 50s	Final period: 51-55	50s and beyond
Menstrual cycles	Variable	Regular	Regular	Cycle length vary increasingly	2 or more skipped periods	Amenorrhea	Amenorrhea
Signs and symptoms	**Dysmenorrhea** Cramping/pain in lower abdomen or pelvic area, bloating, diarrhea, constipation, nausea, vomiting, unusual fatigue, headaches, breast pain/swelling, scanty blood flow, missed work	**Dysmenorrhea:** as previous **Secondary dysmenorrhea (31 yrs.–menopause):** Pain lasts longer; may begin a few days before menses; lasts longer; pain worsens as menses continues; may not go away as it continues, may be caused by other problems like endometriosis, uterine fibroid, ovarian cysts **Premenstrual syndrome (PMS)/premenstrual tension (PMT):** 5 days before menstrual cycle ending within 4 days after period starts Physical, mental, and emotional Over 300 symptoms: most commonly assessed are irritability, tension, depression, bloating, painful and sore breasts, headache fatigue, changes in sexual desire **Premenstrual dysphoric disorder (PMDD):** between 1 and 14 days or longer		Hot flashes, irritability, sleep disturbances, bone loss begins	Same as previous	Vaginal dryness, hot flashes can persist (for some into their 60s and 70s); bone loss progresses, etc.
	Fertility progressively declining						

https://www.acog.org/Patients/FAQs/Dysmenorrhea-Painful-Periods. Accessed January 20, 2019.

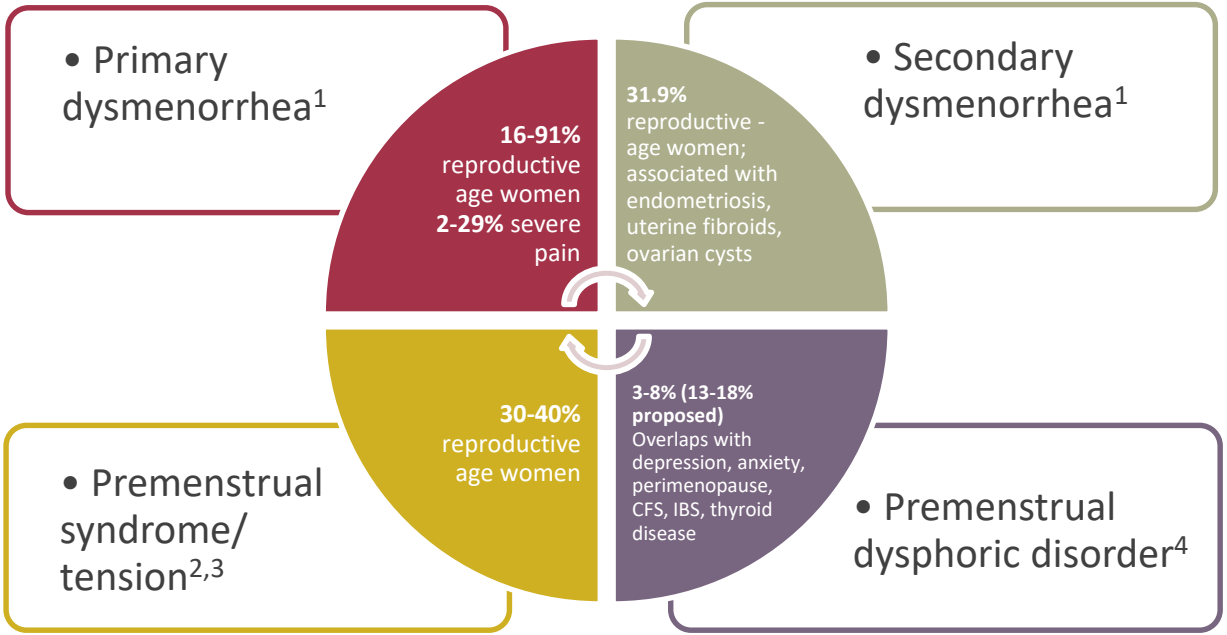
https://www.womenshealth.gov/menstrual-cycle/premenstrual-syndrome. Accessed January 20, 2019.

Kahyaoglu Sut H et al. *Saf Health Work*. 2016;7(1):78-82.

Halbreich U et al. *Psychoneuroendocrinology*. 2003;28 Suppl 3:1–23.

Metagenics Institute

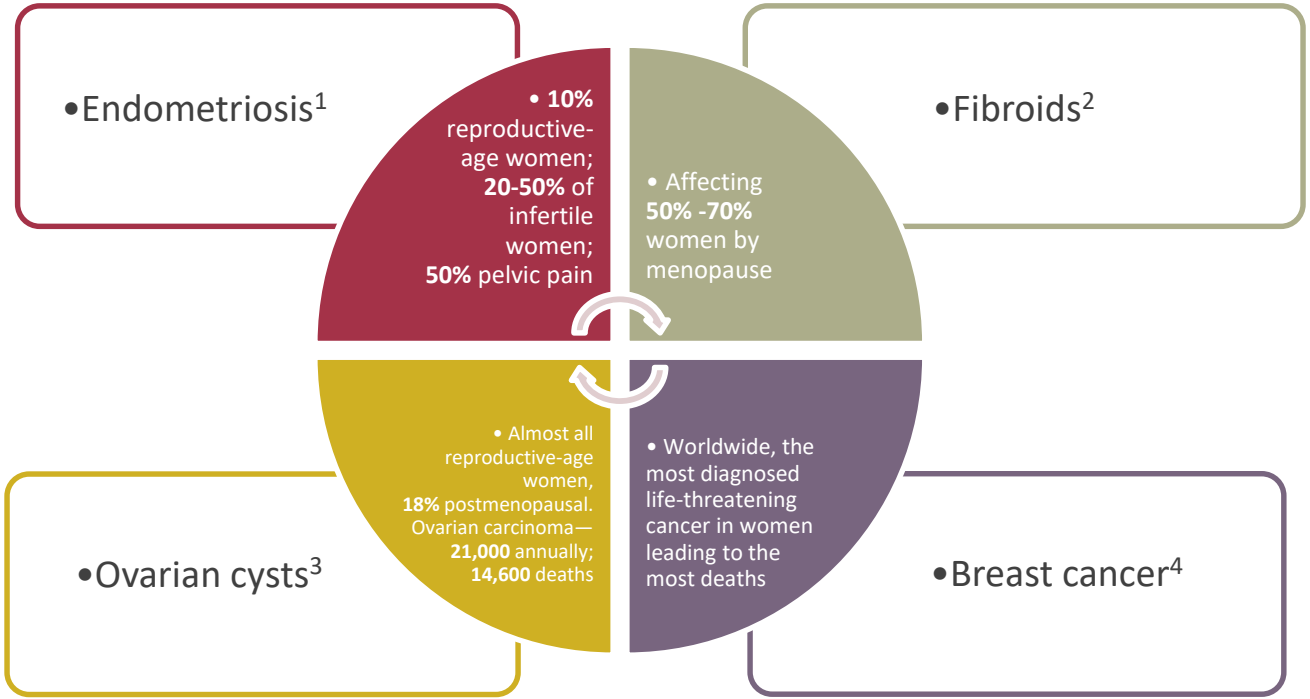
The Quality of Women's Lives, Activities of Daily Living, Emotional Wellbeing, and Other Disorders Are Connected to Shifting Hormones



1. https://www.acog.org/Patients/FAQs/Dysmenorrhea-Painful-Periods. Accessed January 20, 2019.
2. https://www.womenshealth.gov/menstrual-cycle/premenstrual-syndrome. Accessed January 20, 2019.
3. Kahyaoglu Sut H et al. *Saf Health Work*. 2016;7(1):78-82.
4. Halbreich U et al. *Psychoneuroendocrinology*. 2003;28 Suppl 3:1–23.

Metagenics Institute

Morbidity and Fertility Are Also Linked with Hormonal Fluctuations



1. Liang Y et al. *Mol Cell Endocrinol*. 2016;424:42-49.
2. Borahay MA et al. *Reprod Sci*. 2017;24(9):1235-1244.
3. Farghaly SA. *Clin Exp Obstet Gynecol*. 2014;41(6):609-612.
4. Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.

Metagenics Institute

Conventional Approaches Are Palliative and Not Without Side Effects


NSAIDS


Antidepressants


Anxiolytics


Diuretics


Contraceptives/hormones


Surgery

Dietz BM et al. *Pharmacol Rev.* 2016;68(4):1026–1073.
Halbreich U et al. *Psychoneuroendocrinology.* 2003;28 Suppl 3:1–23.
https://www.acog.org/Patients/FAQs/Dysmenorrhea-Painful-Periods. Accessed January 21, 2019.

Metagenics Institute

Conventional Approaches Are Palliative…

Conditions	Interventions
Premenstrual syndrome (PMS), dysmenorrhea (primary and secondary), endometriosis—pelvic pain, IBS	Nonsteroidal anti-inflammatory drugs (NSAIDs)
Premenstrual dysphoric disorder (PMDD) overlapping with affective disorders and perimenopause, sleeplessness	• Selective serotonin reuptake inhibitors (SSRIs) • Serotonin and norepinephrine reuptake inhibitors (SNRIs) • Dopamine agonists • Tricyclic antidepressants (TCA) • Monoamine oxidase inhibitors (MAOIs)
Anxiety, muscle relaxation, insomnia—PMDD, sleeplessness	Benzodiazepines and nonbenzodiazepines
PMS with swollen and painful breasts as dominant symptoms; dysmenorrhea (primary and secondary)	Diuretics to reduce fluid buildup
Dysmenorrhea (primary and secondary), endometriosis	Combined oral contraceptives (OCs)—estrogen (estradiol) and progestogen (progestin)
PMS, menopausal transition, endometriosis	Hormones—natural, synthetic single or combined PMS, menopausal transition, endometriosis estrogen and progesterone (anxiety)
Endometriosis, uterine fibroids, ovarian cysts, breast cancer	Surgery
PMS Dysmenorrhea (primary and secondary)	Calcium, magnesium, vitamin B_6
All disorders	

Dietz BM et al. *Pharmacol Rev.* 2016;68(4):1026–1073.

Halbreich U et al. *Psychoneuroendocrinology.* 2003;28 Suppl 3:1–23.

https://www.acog.org/Patients/FAQs/Dysmenorrhea-Painful-Periods. Accessed January 28, 2019.

Metagenics Institute

Medical Interventions Are Not Without Side Effects

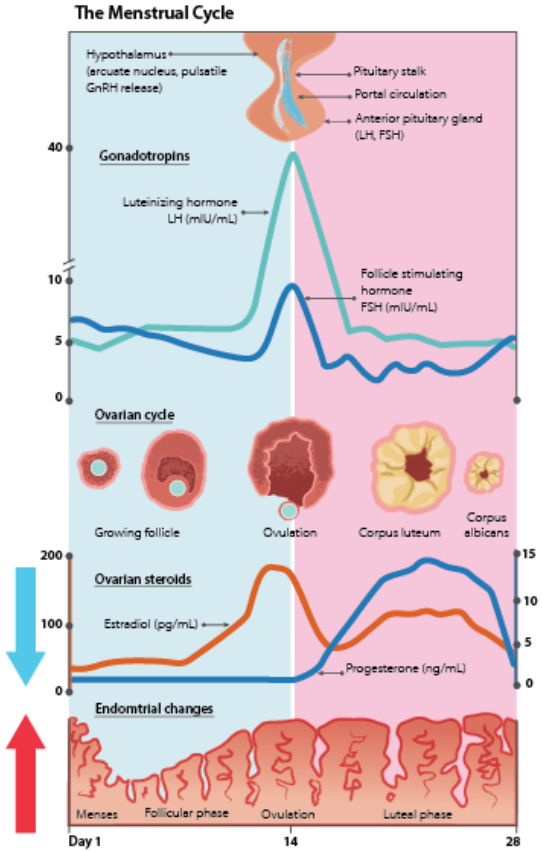
Medication	Side Effects	Targets
NSAIDS	Bleeding, ulcers, vomiting, and diarrhea	Reduce myometrial activity by inhibiting prostaglandin synthesis and reducing vasopressin secretion
Antidepressants	Headache, nausea, insomnia, fatigue, diarrhea, dizziness, sexual side effects, and reduced concentration	Neurohormones: dopamine agonists, selective serotonin reuptake inhibitors, and dopamine/serotonin, monoamine oxidase inhibitors
Benzodiazepines	Dependence, rebound anxiety, memory impairment, and discontinuation syndrome	Gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor
Diuretics	Based on frequency and drug: increased urination, mineral loss (potassium) or excess potassium, low blood sodium (hyponatremia), dizziness, headaches, dehydration, muscle cramps	Calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs) or beta-blockers
Hormones - Oral contraceptives (OC) - Hormone replacement therapy (HRT)	OC: nausea, water retention, and weight gain. Birth control pills may not be suitable for some women, especially those wanting to become pregnant HRT: breast pain, headache or dizziness, weight gain, extremity swelling, vaginal bleeding	OC: suppress ovulation and thin the endometrial lining, which reduces menstrual fluid volume along with prostaglandin levels—decreasing pain (uterine contractions) HRT: address menopausal symptoms, including hot flashes and vaginal discomfort; prevent bone loss in postmenopausal women
Surgery	Infertility, disfigurement, symptoms related to reproductive organ removal	Ovaries, breasts, uterus, endometrium

Dietz BM et al. *Pharmacol Rev.* 2016;68(4):1026–1073.
www.mayoclinic.org/diseases-conditions/menopause/in-depth/hormone-therapy/art-20046372. Accessed March 6, 2019.

Current Conventional Strategies Reveal Complex Interactions That Can Contribute to Symptoms

Understanding the numerous, crucial interactions among individual biochemical, neuroendocrine, and detoxification systems is of key importance to craft a clinical approach.

Metagenics Institute



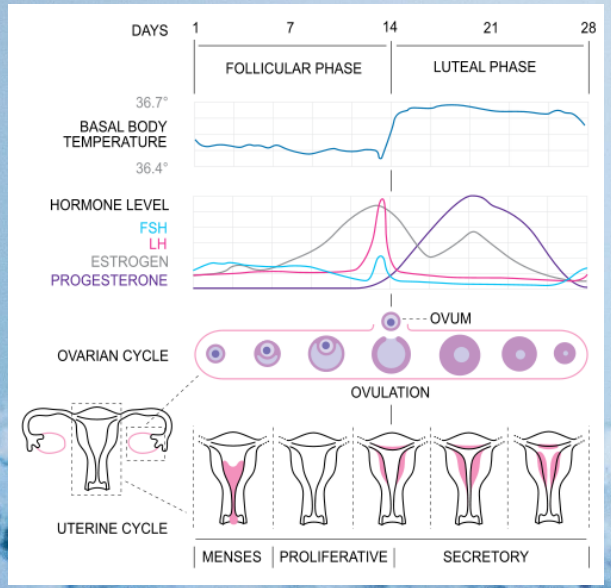
Adapted from: Braverman PK et al. *Pediatr Rev.* 1997;18(1):18.

Clinical Observation:

Premenopause through perimenopause complaints appear to be associated with dramatic fluctuations in the levels of progesterone (P) and estradiol (E), among other hormones affecting the body as a whole, including the central nervous system (CNS).[1-2]

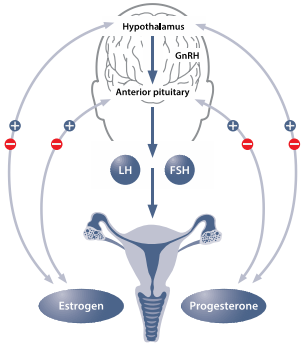
1. Hawkins SM. *Ann N Y Acad Sci*. 2008;1135:10-18.
2. Gray SH. *Pediatr Rev*. 2013;34(1):6-17.

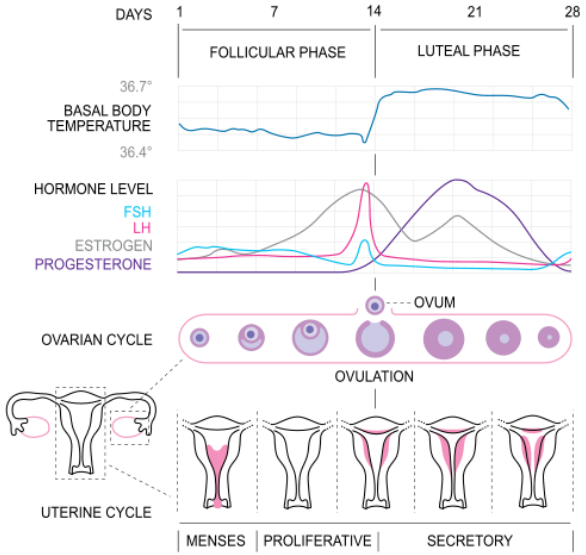
Metagenics Institute



https://commons.wikimedia.org/wiki/File:MenstrualCycle2_en.svg. Accessed February 4, 2019.
https://creativecommons.org/licenses/by-sa/3.0/legalcode

Hypothalamic-Pituitary-Gonadal (HPG) Axis Governs the Menstrual Cycle





Day 1-14 Follicular phase: Estrogen predominant—growth and development

Day 14 GnRH surge generator: Preovulatory surge of GnRH occurring several hours prior to LH surge

Day 14 Luteal surge: Mature follicle released for fertilization, marking the transition from estrogen dominance to progesterone dominance

Day 15-28 Luteal phase: Progesterone rises due to follicular luteinization, and the corpus luteum is formed continuing to secrete progesterone and estrogens, which further inhibits follicular development

No fertilization: Endometrial lining impacted by inflammatory prostaglandins resulting in menses; progesterone decline

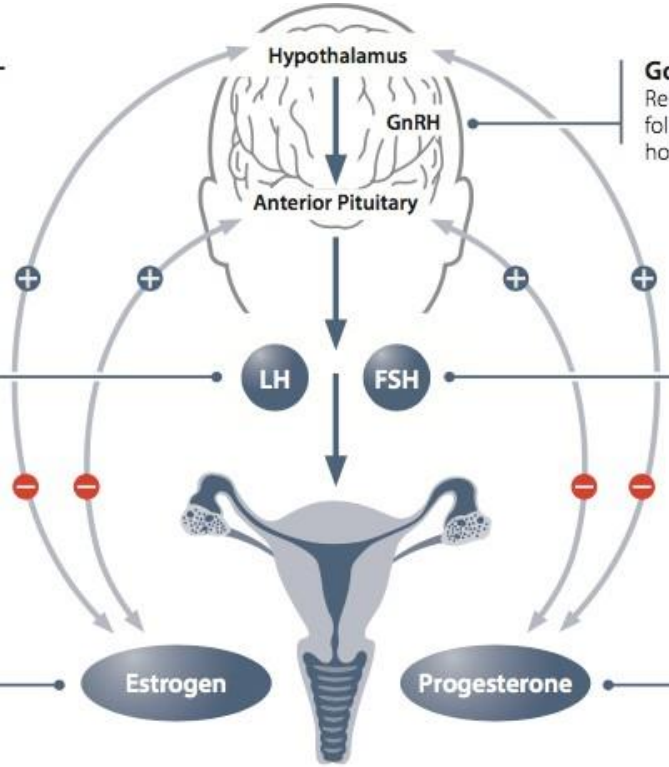
Day 28 Menstruation: Discharge of unused endometrium; progesterone and estrogen low

https://commons.wikimedia.org/wiki/File:MenstrualCycle2_en.svg. Accessed February 4, 2019.
https://creativecommons.org/licenses/by-sa/3.0/legalcode

Hawkins SM. *Ann N Y Acad Sci.* 2008;1135:10-8.

Metagenics Institute

Hypothalamic-pituitary-gonadal (HPG) axis



Gonadotropin-releasing hormone (GnRH)
Releasing hormone responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

Luteinizing hormone (LH)
Functions with follicle-stimulating hormone and stimulates the release of an egg from the ovary. LH is controlled by the hypothalamus and pituitary gland.

Follicle-stimulating hormone (FSH)
Functions with luteinizing hormone and stimulates the release of eggs from the ovaries. It is controlled by the hypothalamus and pituitary gland.

Estrogen
A hormone that affects libido, mood, joints, and mental state. It plays a role in breast tenderness, cysts, cancer, fibroids, endometriosis,endometrial cancer, hot flashes, and symptoms in women who are experiencing or have experienced menopause.

Progesterone
Released in your ovaries, this hormone can be converted into cortisol in the adrenals. A low amount of progesterone can cause anxiety, night sweats, sleeplessness, and irregular cycles.

Plant TM. *J Endocrinol*. 2015;226(2):T41-T54.

Hormonal Imbalances Within the HPG Axis During Menses Can Produce Physical and Mental Distress

- Types of imbalances
  - Estrogen (E) and progesterone (P) hormonal fluctuations within cycle are more significant in relation to symptom severity and condition than absolute hormone levels
  - Lack of ovarian responsiveness to LH and FSH—low E and P
  - Poor corpus luteum integrity—low P
  - Insufficient follicle development—low E and P
  - Imbalance between progesterone and LH pulsatile and amplitude patterns
  - Imbalances between E and P production influence mood, cognition, behavior, and risk of hormone-related illness

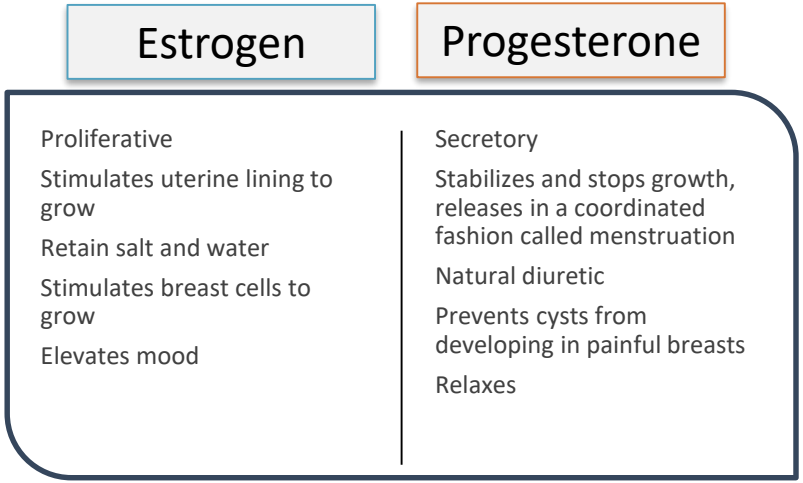
Del Río JP et al. *Front Public Health.* 2018;6:141.
Halbreich U et al. *Psychoneuroendocrinology.* 2003;28 Suppl 3:1–23.

Estrogen and Progesterone Have Counter-Regulatory Effects on Estrogen-Sensitive Tissues

Hormone	Function	Effect
Estrogen (E)[1]	• Excites, stimulates • Regulation of the female reproductive system and secondary sex characteristics	• Fertility • Through receptors influences the endometrium, uterus, mammary gland, cardiovascular system, central nervous system, and bones
Progesterone (P)[2]	• Relaxes, inhibits • Secretory • Plays important roles in the menstrual cycle and in maintaining the early stages of pregnancy	• Fertility and pregnancy maintenance[3] • Through receptors influences the endometrium, uterus, mammary gland, cardiovascular system, central nervous system, and bones

1. Patel S et al. *Biomed Pharmacother*. 2018;102:403-411.
2. Taraborrelli S. *Acta Obstet Gynecol Scand.* 2015;94(Suppl 161):8-16.
3. Di Renzo GC et al. *Horm Mol Biol Clin Investig*. 2016;27(1):35-48.

Estrogen and Progesterone Have Counter-Regulatory Effects on Estrogen-Sensitive Tissues[1-2]



1. Taraborrelli S. *Acta Obstet Gynecol Scand*. 2015;94(Suppl 161):8-16.
2. Del Río JP et al. *Front Public Health.* 2018;6:141.

Metagenics Institute

Estrogen Imbalance Can Affect Mood & Cognition

Imbalanced estrogen levels can alter neurotransmitter balance, potentially leading to mood disruption and cognitive dysfunction

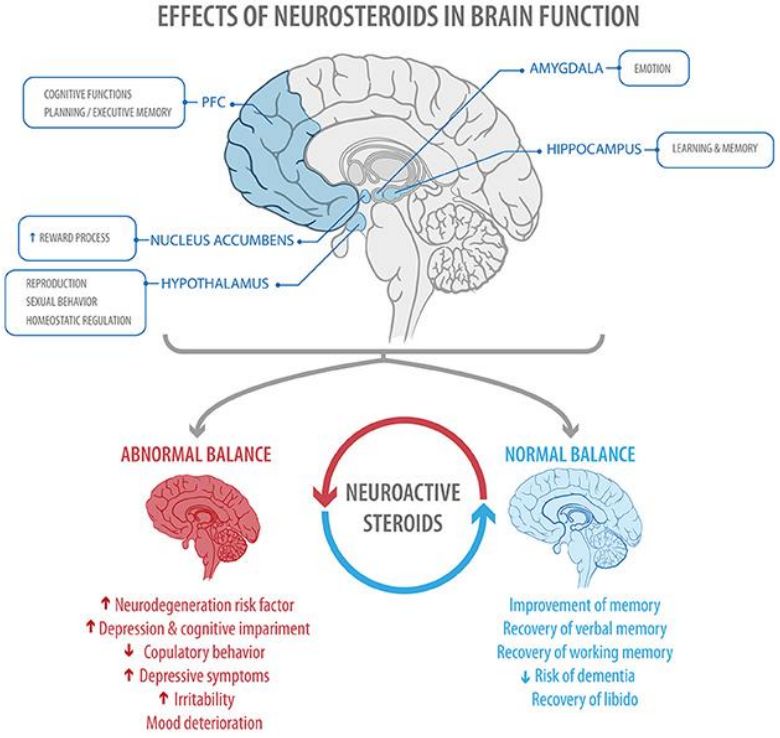


Figure: Del Río JP et al. *Front Public Health.* 2018;6:141. http://creativecommons.org/licenses/by/4.0/. Accessed February 25, 2019.

Metagenics Institute

Conditions Favoring ↑E:P Ratio Affect Mood

↑E suppresses progesterone expression

↑E:P ratio favors an excitatory neurotransmitter shift toward glutaminergic and dopaminergic dominance and GABA suppression, leading to mood disruption

Disruptive conditions:

Prolonged stress exposure (cortisol)

Metabolic concerns: ↑insulin

Insensitivity (MetS), obesity

Chronic inflammatory signals

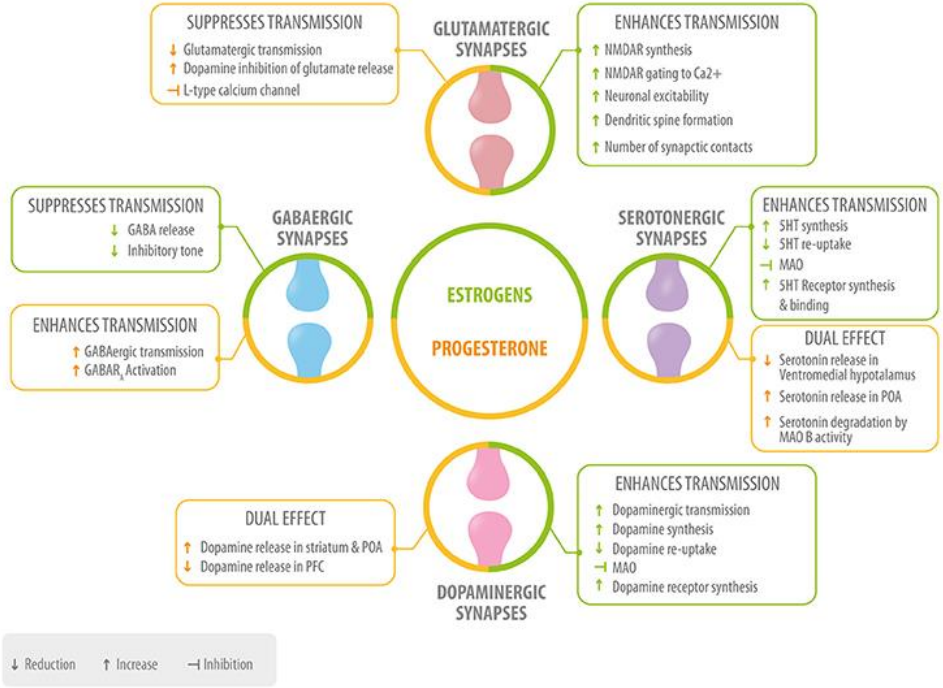


Figure: Del Río JP et al. *Front Public Health.* 2018;6:141. http://creativecommons.org/licenses/by/4.0/. Accessed February 25, 2019.

Metagenics Institute

Cortisol and Insulin—A Reciprocal Relationship

Inflammatory messages signal threat, followed by the release of cortisol, glucose, and insulin

Cortisol → insulin release

Chronically elevated cortisol due to stress → insulin resistance[1]

Insulin resistance associated with inflammation[2]

Inflammation → aromatase activity[3,4]

Aromatase → estrogen levels[5]

1. Joseph JJ et al. *Ann N Y Acad Sci*. 2017;1391(1):20-34.
2. Zand H et al. *Diabetes Metab Syndr*. 2017;11 Suppl 1:S307-S309.
3. Zahid H et al. *Curr Opin Pharmacol*. 2016;31:90-96.
4. Morris PG et al. *Cancer Prev Res (Phila)*. 2011;4(7):1021-1029.
5. Wang X et al. *J Steroid Biochem Mol Biol*. 2015;153:35-44.

KEY TAKEAWAYS

Imbalances between E and P production may contribute to HPG axis imbalance and impact other nonreproductive, hormone-sensitive tissues like the cardiovascular system and bone

Progesterone is an important counter-regulatory hormone to estrogen that acts to oppose estrogen's stimulatory influences on estrogen-sensitive tissues

↑E:P ratio can suppress expression of progesterone leading to hyperproliferation, inflammation, excitability

↑E:P ratio is associated with:

- ↑insulin and blood sugar levels[1]
- immune activation[2]
- ↑inflammation[3]
- ↑aromatase, which increases estrogen formation[4,5]

1. Joseph JJ et al. *Ann N Y Acad Sci*. 2017;1391(1):20-34.
2. Hurwitz A et al. *Endocrinology*. 1991;129(3):1250-1256.
3. Rohleder N. *Psychosom Med*. 2014;76(3):181-189.
4. Wang X et al. *J Steroid Biochem Mol Biol*. 2015;153:35-44.
5. Zahid H et al. *Curr Opin Pharmacol*. 2016;31:90-96.

Excess Estrogen Burden May Be a Prime Contributor to Chronic HPG Axis Imbalance

It is now well known that one of the most prominent causes of breast cancer, as well as many other hormone-related health problems, is excessive estrogen exposure from both **endogenous*** and **exogenous*** sources.[1-3]

*Endogenous estrogens and their metabolites are made by hormonal and nonhormonal tissues. Exogenous estrogens (xenoestrogens) and their metabolites come from environmental sources.[1-3]

1. Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
2. Hua H et al. *Exp Hematol Oncol.* 2018;7:24.
3. Tsuchiya Y et al. *Cancer Lett.* 2005;227(2):115–124.

Metagenics Institute

Endogenous Estrogens

- Estrogens readily diffuse across the cell membrane
- Inside the cell, they bind and activate estrogen receptors

Estrone (E1)

- Weak form of estrogen
- Found in women after menopause
- Small amounts found in most tissues (mainly fat and muscle)
- Body can convert estrone to estradiol and estradiol to estrone

Estradiol (E2)

- Strongest form of estrogen
- Produced by ovaries
- Main contributor to gynecological issues
  - Endometriosis
  - Fibroids
  - Cancers (particularly endometrial cancer)

Estriol (E3)

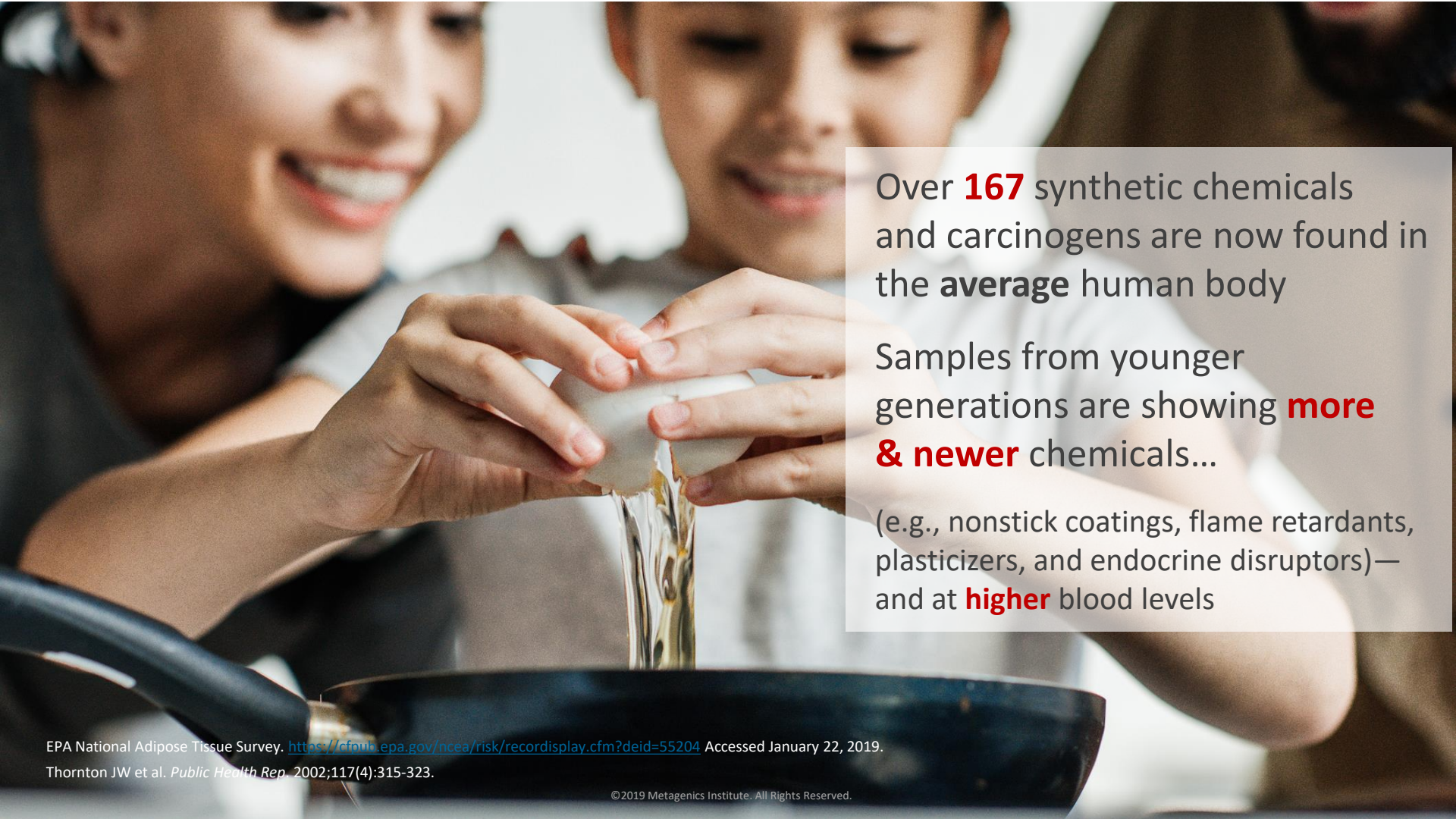
- Weakest of estrogens
- Waste product after body utilizes estradiol
- Peripheral metabolite of E1 and E2
- Significant amounts only produced during pregnancy
- Not made in ovaries
- Cannot be converted to either estradiol or estrone

Farzaneh S et al. *Sci Pharm*. 2016;84(3):409–427.
https://www.hormone.org/hormones-and-health/hormones/estrogen. Accessed January 20, 2019.

Exogenous Estrogens Are Called Endocrine-Disrupting Chemicals (EDCs)

An endocrine-disrupting chemical has been defined by the US Environmental Protection Agency (EPA) as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.”

Diamanti-Kandarakis E et al. *Endocr Rev*. 2009;30(4):293–342.



Over **167** synthetic chemicals and carcinogens are now found in the **average** human body

Samples from younger generations are showing **more & newer** chemicals…

(e.g., nonstick coatings, flame retardants, plasticizers, and endocrine disruptors)—and at **higher** blood levels

EPA National Adipose Tissue Survey. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=55204 Accessed January 22, 2019.

Thornton JW et al. *Public Health Rep.* 2002;117(4):315-323.

Dirty Dozen **Endocrine-Disrupting Chemicals**

1. BPA
2. Dioxin
3. Atrazine
4. Phthalates
5. Perchlorate
6. Fire retardants
7. Lead
8. Arsenic
9. Mercury
10. Perfluorinated chemicals
11. Organophosphate pesticides
12. Glycol ethers

https://www.who.int/ceh/publications/endocrine/en/ Accessed March 5, 2019.
https://www.ewg.org/research/dirty-dozen-list-endocrine-disruptors Accessed January 22, 2019.

Conditions Related to Disruptions in Estrogen Metabolism and Estrogen Dominance[1-4]

- Premenstrual syndrome
- Premenstrual dysphoric disorder
- Fibrocystic or painful breasts
- Dysmenorrhea
- Uterine or ovarian cancer
- Endometriosis
- Uterine fibroid tumors
- Cervical dysplasia
- Systemic lupus erythematosus

1. Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
2. Hua H et al. *Exp Hematol Oncol.* 2018;7:24.
3. Tsuchiya Y et al. *Cancer Lett.* 2005;227(2):115–124.
4. Raftogianis R et al. *J Natl Cancer Inst Monogr.* 2000;27:113–124.

KEY TAKEAWAY

Emerging science strongly suggests that the **combined effects** of endogenous and endocrine-disrupting chemicals (EDCs) can contribute to a state of estrogen dominance.

The Path Less Traveled

Improved estrogen metabolism and clearance can reduce estrogen dominance and promote HPG axis balance by influencing

- Estrobolome: gut-estrogen connection
- Estrogen detox & clearance
- Estrogen-binding & estrogen-receptor sensitivity
- Body's systemic pool of estrogen
- Production & conversion of estrogen

Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
Borahay MA et al. *Mol Med*. 2015;21:242–256.
Ziegler RG et al. *Steroids*. 2015; 99(Pt A):67–75.

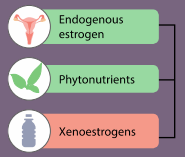
Fourfold Strategy to Improve Estrogen Biotransformation and Clearance

I. Reduce estrogen burden

- Reduce peripheral production via aromatase inhibition
- Reduce exposure to environmental estrogen imitators

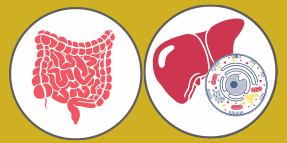
II. Modulate transport

- Sex hormone binding globulin (SHBG)
- Distribution
- Interaction with other hormones



III. Modulate receptors

- Defend cellular sensitivity (estrogen receptors) from other hormones



IV. Support metabolite detox

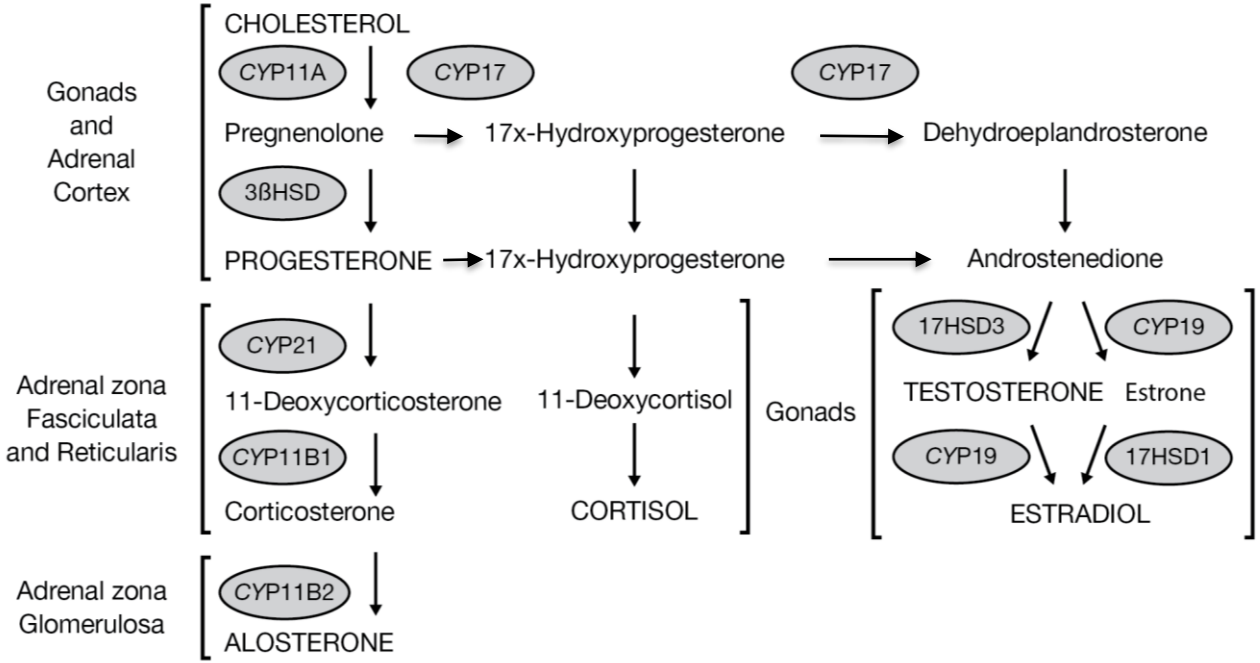
- Promote balanced Phase 1 & Phase 2 estrogen biotransformation and clearance
- Promote a healthy gut microbiome, gut ecology

Metagenics Institute

I. Reduce Estrogen Burden

- Reduce peripheral production via aromatase inhibition
- The estrobolome: promote a healthy gut microbiome, gut ecology
- Reduce exposure to EDCs

Cytochrome P450 and Steroid Hydroxylase Enzymes Synthesize Cholesterol, the Precursor of All Steroid Hormones, to Form Estrogens, Progesterone, Cortisol, and Androgens

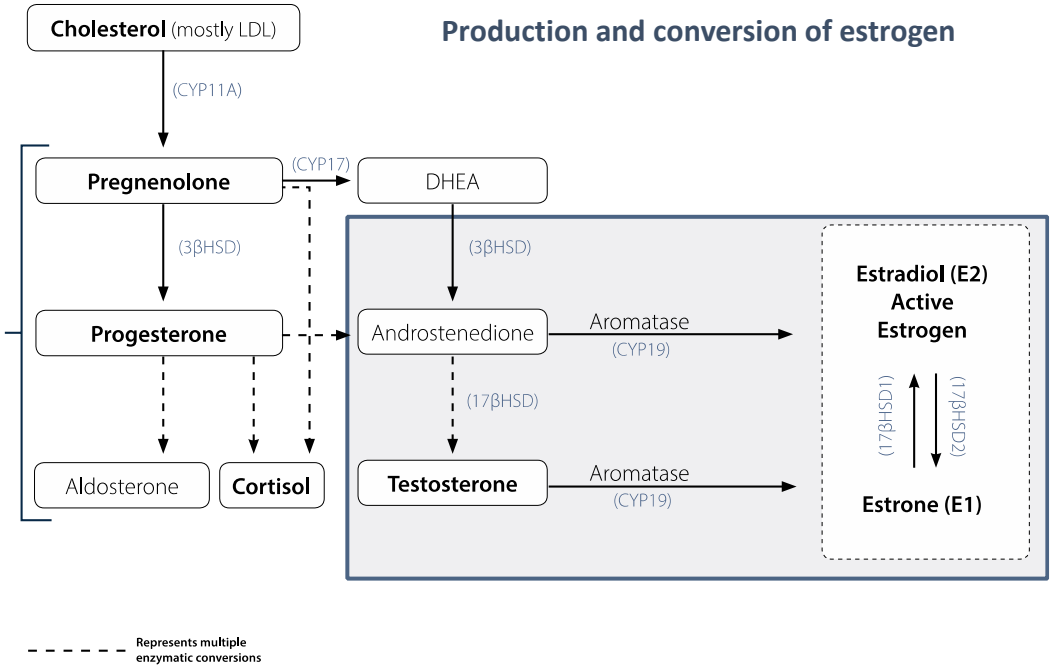


Adapted from: Payne AH et al. *Endocr Rev.* 2004;25(6):947–970.

Miller WL et al. *Endocr Rev.* 2011;32(1):81–151.

Metagenics Institute

Manage Aromatase (CYP19) and Control Estrogen Biosynthesis



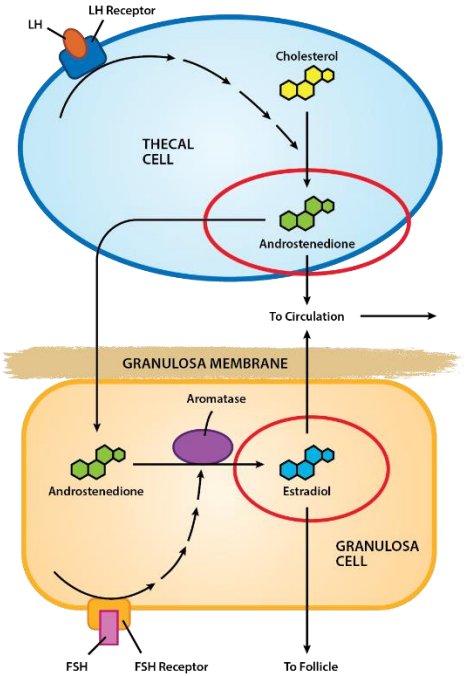
PRODUCTION:
Aromatase (CYP19) transforms androstenedione and testosterone to E1 and E2

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

Metagenics Institute

Premenopause: Aromatase (CYP19) Is Critical to Ovarian Follicle Maturation, Estrogen Production, and the Luteal Surge

- Mature follicle **theca cells** provide **androstenedione**, an estrogen precursor, to the granulosa cells, where androstenedione is **aromatized** (CYP19) to **estrogen**
- **Granulosa cells** transform androstenedione to estrogen (the **"estrogen boost"**) that triggers the pituitary signal to transition to the luteal phase via **luteal surge**

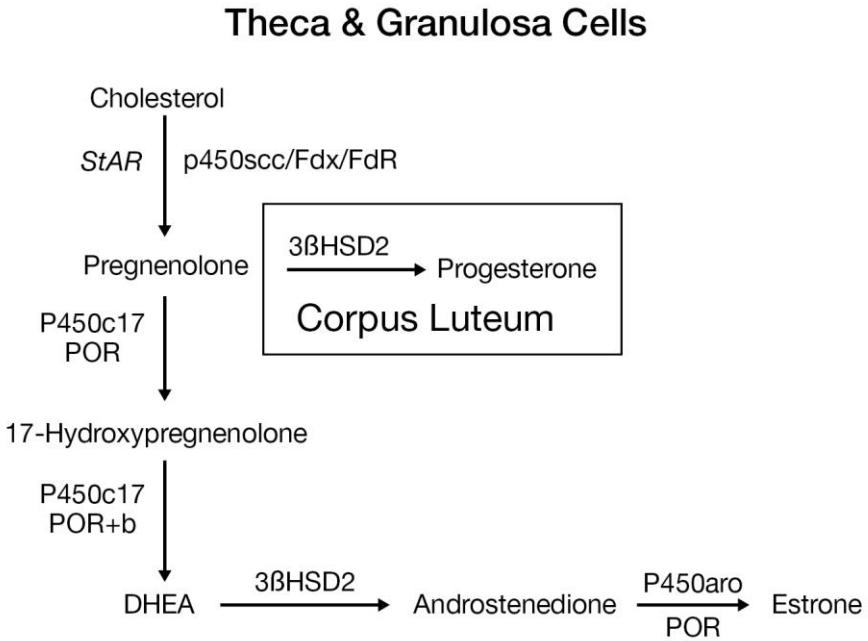


Adapted from: http://what-when-how.com/acp-medicine/amenorrhea-part-1/. Accessed February 25, 2019.

Del Río JP et al. *Front Public Health.* 2018;6:141.

Premenopause: Progesterone Synthesis Requires a Healthy Ovarian Follicle and Corpus Luteum

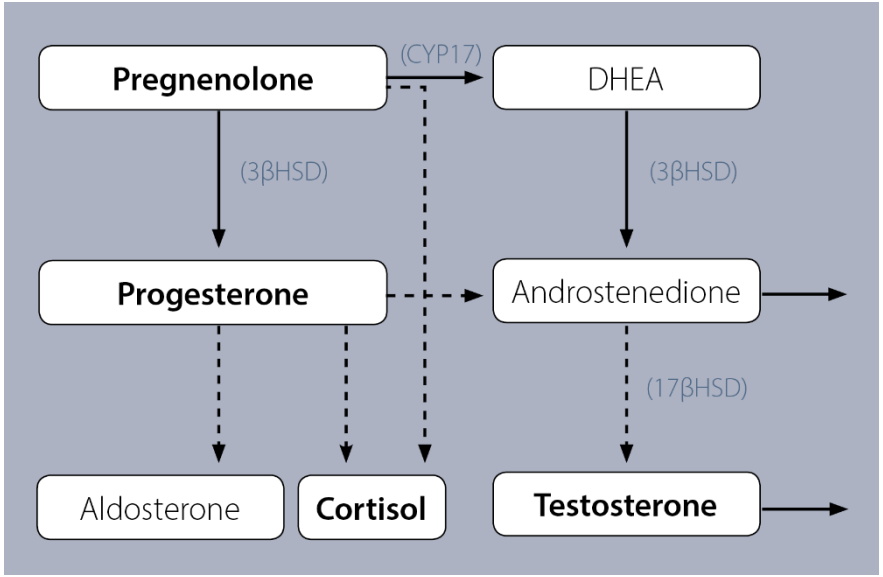
- Corpus luteum is the protective covering of the ripe egg
- When the egg is not fertilized, it is discharged; the corpus luteum closes and continues to secrete progesterone, thickening the endometrial lining, followed by menstruation
- After ovulation, progesterone is made by granulosa cells under stimulation of LH
- Progesterone synthesis ends with menopause



Adapted from: Miller WL et al. *Endocr Rev.* 2011;32(1):81–151.

Miller WL et al. *Endocr Rev.* 2011;32(1):81–151.
Payne AH et al. *Endocr Rev.* 2004;25(6):947–970.

The “Pregnenolone-” or “Cortisol Steal”



Increased demand for cortisol (i.e., chronic stress) reduces availability of estrogen precursors DHEA and androstenedione

This may result in reduced levels of pregnenolone and progesterone, whereby…

E:P ratio increases

Miller WL et al. *Endocr Rev.* 2011;32(1):81–151.

Metagenics Institute

Aromatase Is Rate-Limiting Step in Conversion of Androgens in Extra-Gonadal Tissues to Estrogen

Extra-gonadal sources

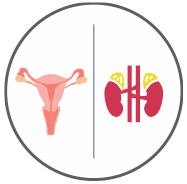
- Adipose tissues
- Bone
- Brain
- Adrenal glands
- Liver
- Pancreas
- Muscles
- Blood vessels
- Skin

Extra-gonadal pathways differ from the ovaries

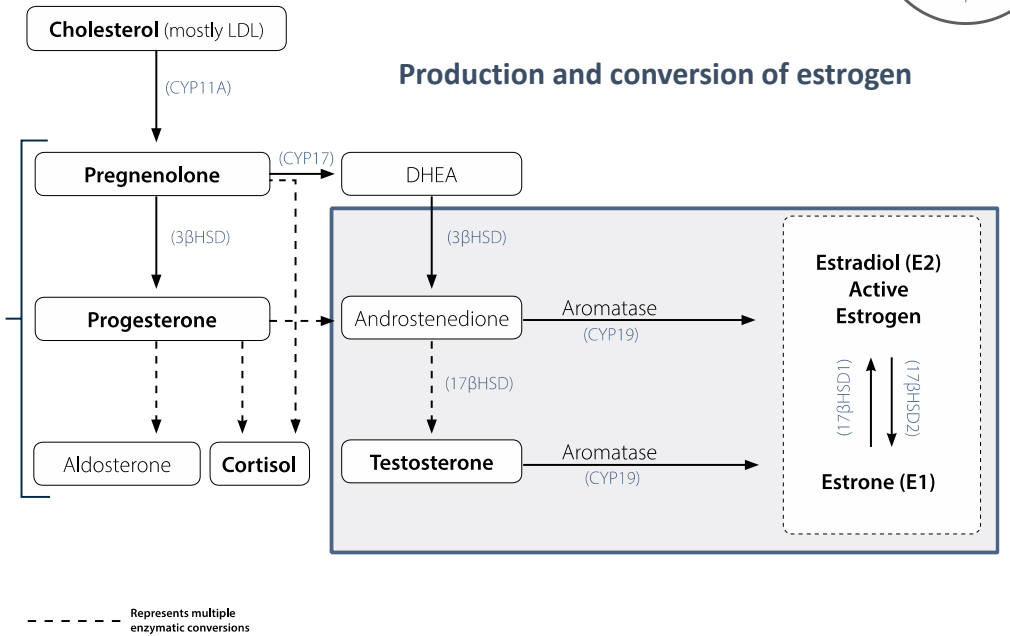
- Unable to synthesize androgens, the precursors of estrogen synthesis
- Can convert androgen steroids to estrogens via a critical and rate-limiting step mediated by CYP19 (aromatase)
- Dependent on an external source of androgen precursors and the level of aromatase expression
- Estrogens metabolized locally limit their systemic effects
- While total tissue estrogen levels may be small, the local tissue concentrations could be high enough to exert biological impact locally

Barakat R et al. *BMB Rep*. 2016;49(9):488-496.

Aromatase Is Stimulated by Inflammation and Contributes to Excess Estrogen Body Burden



- Aromatase is upregulated by prostaglandins[1]
- Responds to positive feedback cycle
- Increased levels of aromatase are found in higher fat-to-muscle ratio
- Increased aromatase levels are linked to:[2]
  - Synovial fluid in rheumatoid arthritis
  - Uterine fibroids
  - Endometriosis
  - Breast cancer cells



1. Richards JA et al. *J Clin Endocrinol Metab.* 2003;88(6):2810-2816.
2. Bulun SE et al. *Sem Repr Med.* 2004;22(1):45.

Metagenics Institute

Slight Reduction of Aromatase Produces Significant Effect

- Extra-glandular aromatase expression, especially in fat, can convert androgens to estrogens[1]
- Insulin sensitivity is important[2]
  - Inhibits the activity of aromatase, which converts testosterone into estrogens
  - Limits conversion of testosterone to estrogen

1. Williams G. *Mol Cell Endocrinol*. 2012;351(2):269-278.
2. Iyengar NM et al. *Cancer Prev Res (Phila)*. 2017;10(4):235-243.

Evidence Shows Obesity and Breast Cancer Linked to Elevated Levels of Aromatase, Inflammation, and Dysregulated Metabolism

- Data show that obesity is associated with increased risk of estrogen-dependent cancers (breast cancer) in postmenopause[1]
- Adipose tissue undergoes many significant changes due to excess lipid storage, which leads to adipocyte cell death and the subsequent recruitment of macrophages[2]
- This results in a chronic, low-grade, inflammatory state correlating to:
  - Activation of NF-κB signaling
  - Elevation of aromatase levels

1. Iyengar NM et al. *Cancer Prev Res (Phila)*. 2017;10(4):235-243.
2. Zahid H et al. *Curr Opin Pharmacol*. 2016;31:90-96.

KEY TAKEAWAYS

Aromatase (CYP19) is a double-edged sword: critical to the production of healthy ovarian function and progesterone as well as excess estrogen synthesis through the aromatization of androstenedione in extra-gonadal tissues.

Postmenopause: Estrogen is produced by extra-gonadal tissues (i.e., adipose tissue) without the balancing influence of progesterone.*

Managing aromatase expression in extra-gonadal tissues by promoting insulin sensitivity, healthy weight, and reduction of prolonged stress can help modulate the estrogen pool.

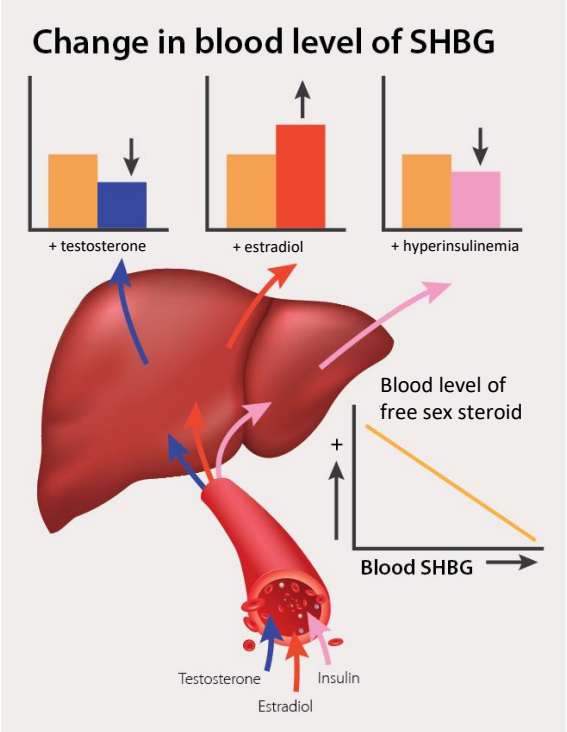
*Adrenal production of progesterone is insignificant

II. Modulate Estrogen Transport

- Sex hormone binding globulin (SHBG)
- Distribution
- Interaction with other hormones

Estrogen Transport: Sex Hormone Binding Globulin (SHBG)

- SHBG is made mostly in the liver and binds to both testosterone and estrogen[1]
  - SHBG controls amount of bioavailability of these hormones in the body
  - SHBG levels are usually twice as high in women than men[2]
- Raising SHBG levels lowers the amount of free hormone (and vice versa)
  - Increasing circulating concentrations of SHBG reduces levels of unbound, active estrogens[3]



Adapted from: https://www.who.int/ipcs/publications/en/ch3.pdf
Accessed March 25, 2019.

1. Hammond GL. *J Endocrinol*. 2016;230(1):R13-25.
2. Handelsman DJ et al. *Ann Clin Biochem*. 2016;53(Pt 3):377-384.
3. Thaler MA et al. *Best Pract Res Clin Endocrinol Metab*. 2015;29(5):749-760.

Metagenics Institute

Clinical Significance of SHBG Levels

30% of SHBG attaches to albumin,[1] so adequate protein intake is critical for hormonal health

SHBG levels increased in:[2]

- Pregnancy
- Hyperthyroidism
- Liver disease
- Exogenous estrogen
  - Hormone replacement therapy (HRT) or oral contraceptives (OC)

SHBG levels decreased in:[3]

- Obesity (breast cancer risk)
- Insulin use
- Polycystic ovary syndrome
- Hypothyroidism
- Androgen use
- Cushing's syndrome (corticoids)

1. Hammond GL. *J Endocrinol*. 2016;230(1):R13-25.
2. Handelsman DJ et al. *Ann Clin Biochem*. 2016;53(Pt 3):377-384.
3. Thaler MA et al. *Best Pract Res Clin Endocrinol Metab*. 2015;29(5):749-760.

Clinical Significance of SHBG Levels

SBGH Level	Clinical Significance
↓ by increased weight gain[1]	Middle-age “spread” or perimenopausal weight gain
↓ by increased visceral adipose tissue (VAT)[2]	Independent of weight gain, VAT is positively associated with hypogonadism
↓ by increased insulin levels; considered a marker for insulin resistance (IR)[3]	Decreased with IR and hyperinsulinemia; low SHBG is a predictor for T2D development

Hormone/Condition	Effect on SBGH[1]
Thyroxine	Increase ↑
Estradiol	Increase ↑
Testosterone	Decrease ↓
Insulin	Decrease ↓
Growth hormone	Decrease ↓
Prolactin	Decrease ↓
Cortisol	Decrease ↓
Progestins (some)	Decrease ↓
Obesity	Decrease ↓

1. Strauss JF et al. *Yen & Jaffe’s Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management*. 4th ed. W.B. Saunders and Co. 1999.
2. Wang N et al. *Sci Rep*. 2016;6:19844.
3. Wallace IR et al. *Clin Endocrinol*. 2013;78(3):321-329.

Metagenics Institute

Modest Weight Loss Leads to Raised SHBG

Study objective: Does weight loss, with or without exercise, lead to beneficial effects on SHBG?
Primary outcomes: Effects on SHBG and sex hormones after 16-week intervention

Demographics/inclusion & exclusions

- 243 women aged 50-69 years
- Postmenopausal ~ 12 mo.
- Inactive (< 2 hrs./wk. of ≥ 4 MET activity)
- BMI 25-35 kg/m^2
- Not using beta-blockers or oral steroids
- Nonsmokers, no drugs or alcohol
- No history of any cancers, diabetes, or other endocrine disorders

Randomized interventions for 16 weeks

Group 1—Diet only (n = 97)

- Calorie restriction 3,500/wk.
- Maintain usual activity levels
- Dietitian contact twice weekly

Group 2—Diet and exercise (n = 98)

- Calorie restriction 1,750/wk.
- 4 hr./wk. aerobic and strength activity

Group 3—Control (n = 48) (usual activities)

Results

- Both Group 1 (-4.9 kg) and Group 2 (-5.5 kg) achieved target weight loss
- Body fat losses significantly greater in Group 2 vs. Group 1 (−1.4 kg; $p < 0.001$)
- When compared to Group 3:
  - Both Groups 1 & 2 had significant increases in estradiol, free estradiol, androstenedione (Group 2 only), and free testosterone

Conclusions

- In postmenopausal women modest 6-7% weight loss leads to:
  - Better body composition and fitness when combined with exercise
  - With diet alone or with exercise there are still greater and beneficial increases in sex hormones (free testosterone, estradiol, and free estradiol) as well as SHBG levels

van Gemert WA et al. *Breast Cancer Res*. 2015;17:120.

Metagenics Institute

KEY TAKEAWAYS

Insufficient SHBG function is related to **poor lifestyle factors**

Body weight gain, visceral fat, and inflammatory state are all associated with **reduced SHBG levels**

Low SHBG results in **greater amounts of circulating estrogen** available to bind ERs in the body

III. Modulate Estrogen Receptors

- Modify estrogen receptor activity
- Defend estrogen receptors from other hormones
- Protect against estrogen-disrupting chemicals

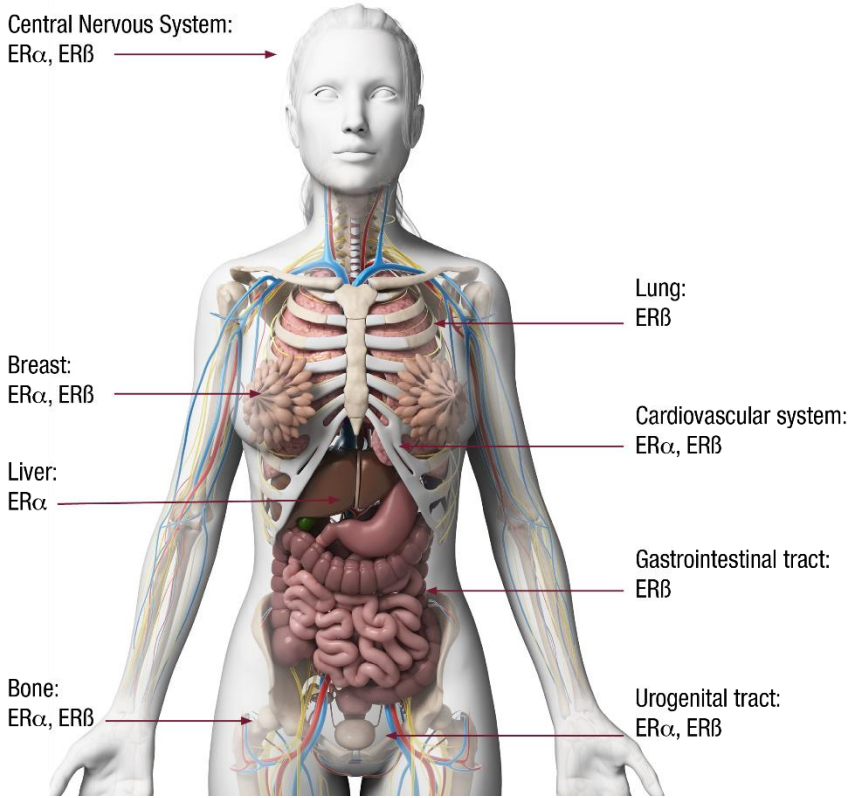
Estrogen's Biological Actions Are Mediated by Estrogen Binding to ERα and ERβ Nuclear Receptors[1-4]

Tissues with *both* ERα and ERβ

- Breast
- Ovary
- Bone
- Central nervous system
- Cardiovascular system
- Uterus

Tissues with *either* Alpha or Beta

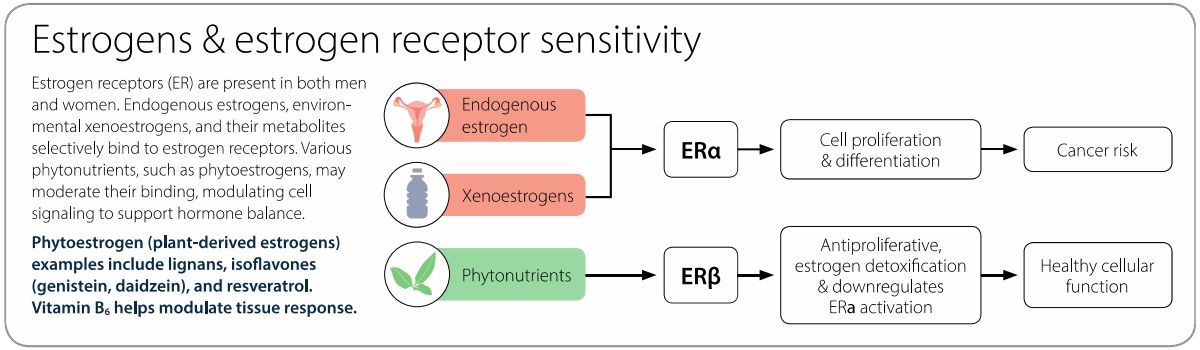
ERα	ERβ
• Liver • White adipose tissue	• Vessels • Lung • Urinary tract • Intestinal lining



1. Dahlman-Wright K et al. *Pharmacol Rev.* 2006;58:773-781.
2. Matthews J et al. *Mol Interv*. 2003;3(5):281-292.
3. Enmark E et al. *Endocr Relat Cancer*.1998;5(3):213-222.
4. Couse JF et al. *Endocrinology*.1997;138(11):4613–4621.

Metagenics Institute

Estrogen Signaling and Binding



ERα[1]

Increases proliferation
Excitatory
Proinflammatory
Increases glycolysis

ERβ[1]

Downregulates ERα activation
Antiproliferative
Estrogen detoxification
Anxiolytic
Anti-inflammatory
Increases brain-derived neurotrophic factor (BDNF)

Xenoestrogens (EDCs), such as bisphenol A (BPA), can bind to both ERα and ERβ.[2]
Many phytoestrogens have a higher binding affinity for ERβ.[3-5]

1. Del Río JP et al. *Front Public Health*. 2018:6:141.
2. Gao H et al. *Medicine (Baltimore)*. 2015;94(1):e211.
3. Harris DM et al. *Exp Biol Med (Maywood)*. 2005;230(8):558-568.
4. Shanle EK et al. *Chem Res Toxicol*. 2011;24(1):6-19.
5. Kuiper GG et al. *Endocrinology*. 1998;139(10):4252-4263.

Metagenics Institute

E:P Ratio Influences Progesterone's Biological Actions and Binding to PR-A and PR-B

Progesterone actions	
Uterus/ovaries	Release of oocytes
	Facilitation of implantation
	Maintenance of pregnancy via myometrial quietening
	Stimulation of stromal regeneration: luteal phase of cycle
Mammary gland	Lobular alveolar development
	Suppression of milk protein synthesis during pregnancy
Brain	Mediation of sexual responsiveness
Bone	Regulation of bone mass; prevention of bone loss

Tissue	Cell type
Uterus	Endometrium
	Myometrium
Ovary	Luteinizing granulosa
	Preovulatory granulosa
	Corpus luteum
Reproductive tissues	Testes
	Vagina
Breast	Normal and neoplastic
Brain	Pituitary
	Ventromedial
	Hypothalamus
	Preoptic area
Other	Vascular endothelium
	Thymus
	Pancreatic islets
	Osteoblast-like cells
	Lung

Al-Asmakh M. *Middle East Fertil Soc J.* 2007;12(3):147-152.

KEY TAKEAWAYS

ERβ downregulates ERα

Premenopausal circulating E2 (estradiol) prefer ERα

Endocrine-disrupting chemicals
(EDCs; xenoestrogens) prefer ERα

Select plant phytochemicals from
vegetables, fruits, and herbs that selectively
bind to ERβ

Reduce exposure to EDCs

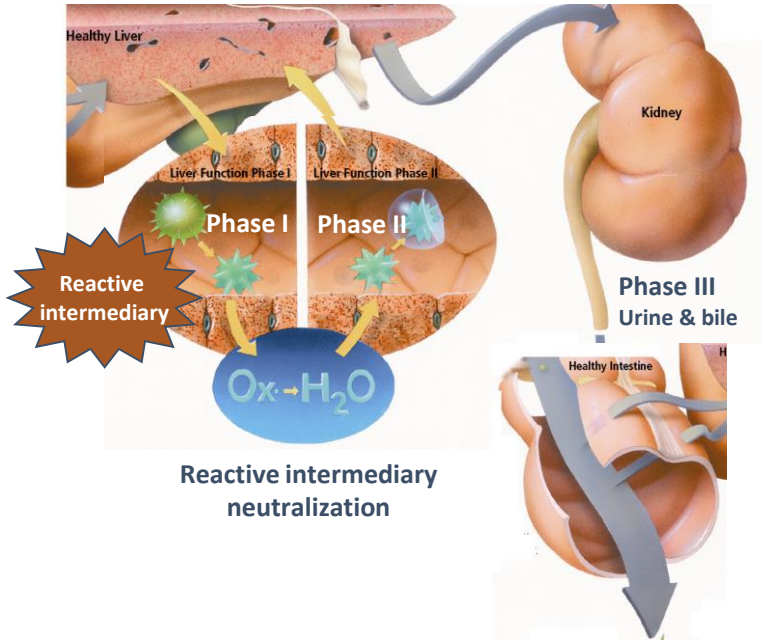
*Adrenal production of progesterone is insignificant in its ability to exert systemic effects

IV. Support Estrogen Detoxification: Biotransformation, Conjugation, and Clearance of Estrogen Metabolites

- Promote balanced and coordinated function between Phase I estrogen bioactivation and Phase II active metabolite conjugation to protect estrogen-sensitive tissues from overstimulation
- Reduce genotoxic estrogen metabolites
- Enhance excretion of estrogen metabolites via coordinated gut-liver function

3 Phases of Detoxification

Body treats estrogen as a toxin



Phase I: Bioactivation—cytochrome P450 superfamily of enzymes (CYP450)

- First defense to biotransform xenobiotics, steroid hormones, and pharmaceuticals
- Located mainly in the liver, but also in enterocytes, kidneys, lung, and even the brain
- CYP450 enzymes generate reactive oxygen species as spin-off products (reactive intermediates)
- Requires oxygen, and often results in a more reactive toxin

Phase II: Conjugation of reactive Phase I metabolites to water-soluble, less reactive compounds

- Catechol-O-methyltransferase (COMT)
- Reduction of oxidized reactive estrogen metabolites through induction of Nrf2 system—quinone reductase, glutathione-S-transferase
- Glucuronidation

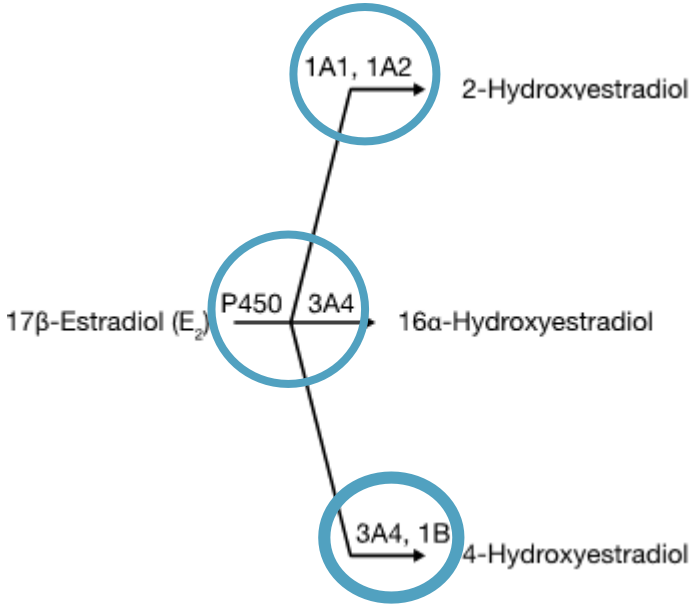
Phase III: Excretion

- Neutralized toxins are removed from the body through bile and urine
- Excretion is aided by a slightly alkaline pH

Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.

Metagenics Institute

Phase I Estrogen Bioactivation—3 Paths and 3 Metabolites



Protective & weak estrogen

Carcinogen & active estrogen

Carcinogen & active estrogen

Promote protective 2-OH (CYP1A1,1A2) over 4-OH and 16α-OH

Estrogen metabolite ratios (↑2-OH:16α-OH) may be more important than total E body burden

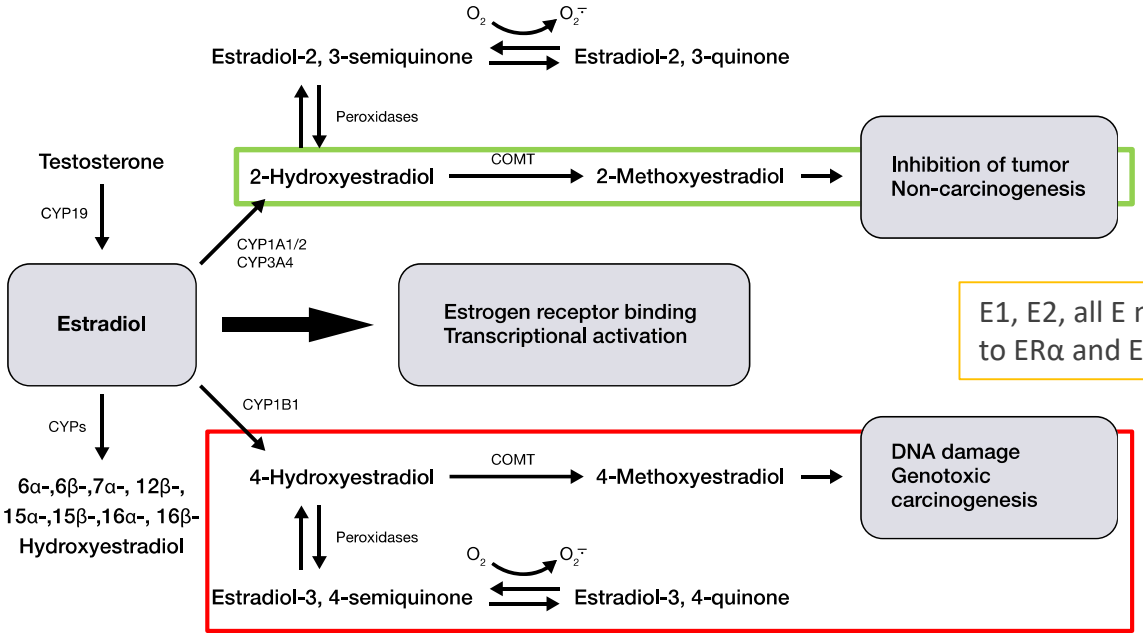
Reduce production of 4-OH by decreasing CYP3A4,1B1

Tsuchiya Y et al. *Cancer Lett.* 2005;227(2):115–124.
Roberts MS et al. *Clin Pharmacokinet*. 2002;41(10):751-790.

Emerging Evidence Supports Associations Among Phase I Estrogen Metabolites, Autoimmunity, Cancer

Year	Publication	Summary & Conclusions
2000	Muti P et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16α-hydroxyestrone ratio in premenopausal and postmenopausal women. *Epidemiology*. 2000;11(6):635-640.	• In a study of 10,786 women it was found that the ratio of 2-OH to 16α-OH was a very sensitive indicator of breast cancer risk • The higher the ratio of 2:16αOH, the lower the incidence of breast cancer
2003	Castagnetta LA et al. Increased estrogen formation and estrogen to androgen ratio in the synovial fluid of patients with rheumatoid arthritis. *J Rheumatol*. 2003;30(12):2597-2605.	• In a study of 20 patients of rheumatoid arthritis (RA) (12 active; 8 control) it was found increased estrogen formation and estrogen to androgen ratio in synovial fluid (SF) of patients with RA • 16α-OH and 4-OH levels raised in RA vs. controls
2009	Schmidt M et al. Estrone/17β-estradiol conversion to, and tumor necrosis factor inhibition by, estrogen metabolites in synovial cells of patients with rheumatoid arthritis and patients with osteoarthritis. *Arthritis Rheum*. 2009;60(10):2913-2922.	• In a study evaluating downstream conversion of estrogens in RA vs. OA in synovial cells it was found that levels of 16α-OH/16α-OH-17β-estradiol were higher than the levels of all other estrogen metabolites • RA synovial cells produced more 16α-OH than did OA synovial cells
2015	Zeigler RG et al. Epidemiologic studies of estrogen metabolism and breast cancer. *Steroids*. 2015;99(Pt A):67-75.	• Novel high-sensitivity LC-MS/MS assay used in three studies shows this assay, which measures all 15 estrogens and estrogen metabolites in human serum and urine, links enhanced 2-OH of parent estrogens to reduced risk of postmenopausal breast cancer; similar pattern seen in premenopausal breast cancer • Associations with ratios of estrogen metabolism pathways are independent of recognized association of unconjugated estradiol with increased risk

Phase II Conjugation of Reactive Estrogen Metabolites



Promote noncarcinogenic 2-methoxyestradiol (2MeOHE) via catechol-O-methyltransferase (COMT)

E1, E2, all E metabolites bind selectively to ERα and ERβ

Reduce oxidation (peroxidation) of 4-hydroxyestradiol (4-OH) to estradiol-3,4-semiquinone, 3,4-quinone and formation of depurinating DNA adducts by inducing Nrf2, quinone reductase (NQO1), heme-oxygenase (HO), glutathione S-transferase (GST) and reduced glutathione (GSH)

Adapted from: Tsuchiya Y et al. *Cancer Lett.* 2005;227(2):115–124.

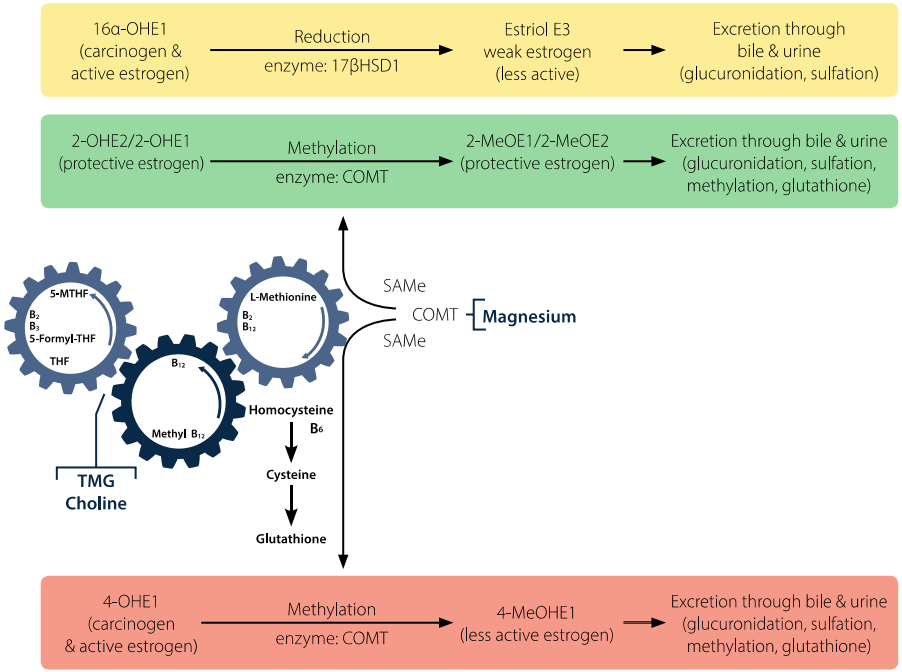
Metagenics Institute

Phase II Conjugation Enzymes	Functions
Catechol-O-methyltransferase (COMT)	Methylate 2-OH to protective 2-MeOHE Discourages formation of DNA damaging depurinating adducts in estrogen-sensitive tissue
Nrf2 (nuclear factor erythroid 2 p45-related factor 2)	Reduces genotoxic estradiol-3,4-quinone Key to regulate body's detoxification and antioxidant systems through gene induction of endogenous antioxidant systems and Phase II enzymes
Glutathione S-transferases (GSTs)	Glutathione conjugation: attaches a glutathione group to a biotransformed metabolite reducing its carcinogenicity Powerful antioxidant: reduced glutathione (GSH) Induced through the production of reactive oxygen species (ROS) via gene transcription involving the antioxidant response element (ARE) and xenobiotic responsive element (XRE)
UDP-Glucuronosyltransferases (UGTs)	Glucuronidation—process that enhances elimination of biotransformed toxins in urine and feces and metabolizes steroid hormones (estrogens) and bilirubin
Sulfotransferases (SULTs)	Incorporates sulfur: active in liver, adrenal, intestine, brain and skin tissues; ↓function by EDCs affecting thyroid, estrogen, and androgen levels

Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.

Phase 2 Methylation

Promote “good” 2-MeOHE via catechol O-methyltransferase (COMT)



This process neutralizes the hydroxylated estrogen metabolites into less active molecules:

Estriol (E3)
2-MeOE1
4-MeOE2

which can be safely eliminated through bile and urine (Phase 3 detoxification)

Metagenics Institute

Methyltransferases: COMT Hypomethylation[1]

- "The conjugating donor compound in methyltransferase reactions is a methionine group from S-adenosyl-L-methionine (SAMe)."[2]
- "Catechol-O-methyltransferase (COMT) is one of the prominent methyltransferases that has received wide attention due to its role in estrogen detoxification"[3]
- "Support for methylation consists of nutrient cofactors and methyl donors, such as methionine, vitamin B_{12}, vitamin B_6, betaine, folate, and magnesium. Various foods can provide these nutrients."[4]
- "Conversely, a high sucrose diet may inhibit methylation enzymes such as COMT."[5]

1. Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.
2. Kohalmy K et al. *Curr Drug Metab*. 2011;12(2):104-123.
3. Yager JD. *Steroids*. 2014;99(Pt A):56-60.
4. Lord RS et al. *Laboratory Evaluations for Integrative and Functional Medicine*. Duluth, GA. 2nd ed. 2012.
5. Busserolles J et al. *Life Sci*. 2002;71(11):1303-1312.

Metagenics Institute

Clinical Insights:

Adverse Health Effects of Poor Folate Metabolism

Health conditions linked to poor folate metabolism[1]

- Coronary heart disease
- Endothelial injury
- Alzheimer's disease
- Stroke
- Down syndrome and miscarriage
- Spina bifida
- Colon and breast cancers
- Schizophrenia and depressive disorders

**Including hypomethylation and hyperhomocysteinemia*

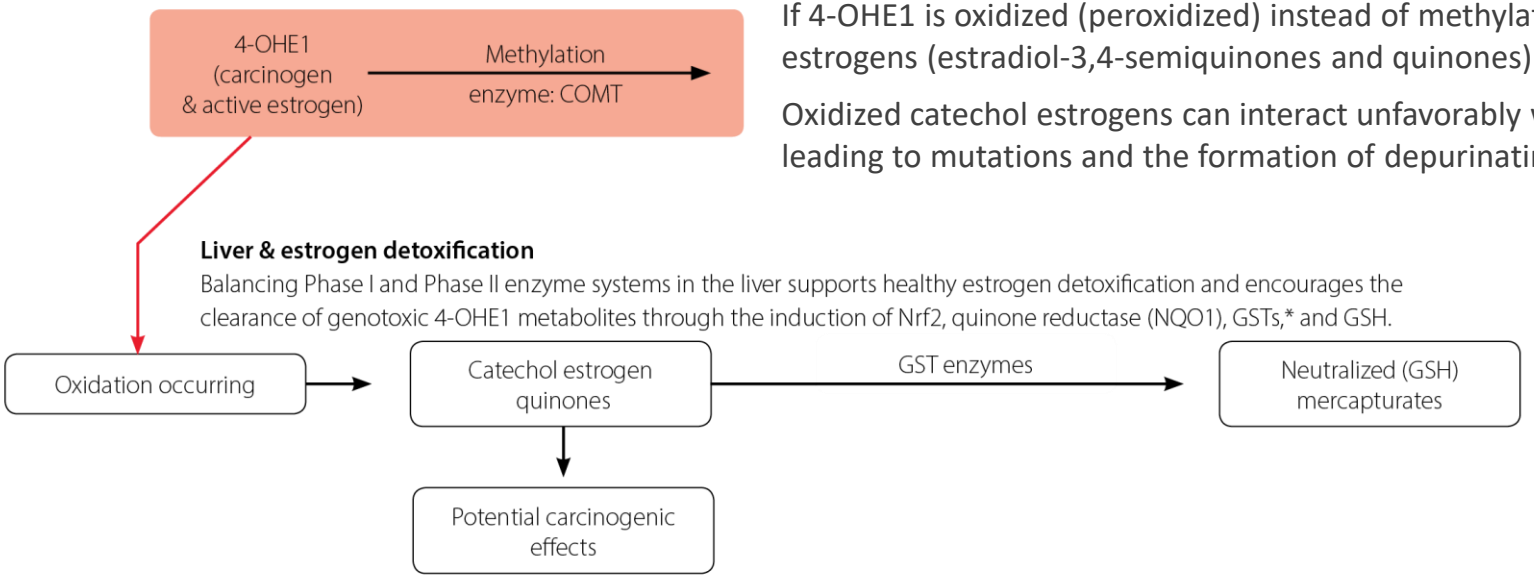
Effects of folate/methylation-related genetic polymorphisms

- Methylenetetrahydrofolate reductase (MTHFR)—C677T[2-3]
  - 10-20% of population have inefficient folate cycle and homocysteine metabolism
- Catechol-O-methyltransferase (COMT)[4]
  - 30% of population have slower COMT activities related to methylation of estrogen detoxification
- Folate polyglutamate hydrolase[5]
  - 10% cannot absorb food folates well

1. Nazki FH et al. *Gene*. 2014;533(1):11-20.
2. Burghardt KJ et al. *Mol Diagn Ther*. 2013;17(1):21–30.
3. Marini NJ et al. *Proc Natl Acad Sci U S A*. 2008;105(23):8055-8060.
4. Hiraoka M et al. *Congenital Anomalies.* 2017;57:142–149.
5. Mitchell ES et al. *Neurosci Biobehav Rev*. 2014;47:307-320.

Metagenics Institute

Phase II Reduction of Catechol Estrogens Via Nrf2 Induction Critical to 4-OH Neutralization



If 4-OHE1 is oxidized (peroxidized) instead of methylated, catechol estrogens (estradiol-3,4-semiquinones and quinones) are produced

Oxidized catechol estrogens can interact unfavorably with DNA, leading to mutations and the formation of depurinating DNA adducts

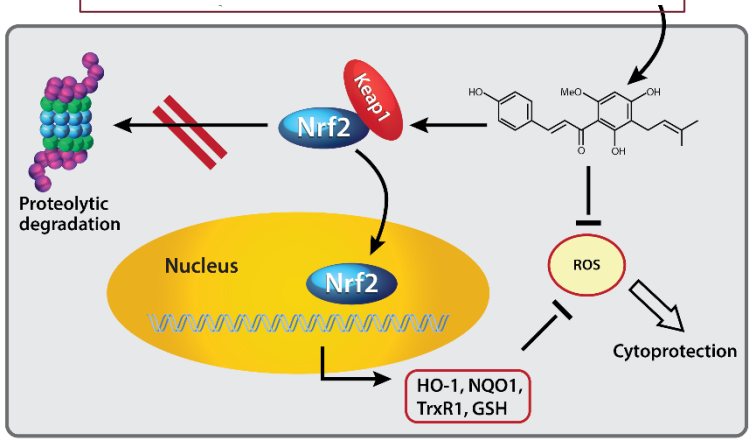
Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
Tsuchiya Y et al *Cancer Lett.* 2005;227(2):115-24.
Cuendet M et al. *J Nat Prod*. 2006;69(3):460-463.

Metagenics Institute

Quinone Reductase (NQO1) as a Biomarker for Cancer Chemoprevention

- Strategies for protecting cells from initiation events
  - *Modulate* metabolic enzymes (i.e. CYP1A1) responsible for generating reactive intermediaries (*Phase I enzymes*)
  - *Increase Phase II enzymes* that can deactivate radicals and electrophiles, like 4-OH, known to intercede in normal cellular processes
- Induction of the Phase II detoxification enzymes via Nrf2 system activation, such as QR, is a useful strategy for cancer chemoprevention
- Quinone reductase (QR/NQO1) reduces electrophilic quinones

Many edible plants have been found to contain cancer chemopreventive agents capable of inducing Phase II enzymes



Adapted from: Yao J et al. *J Agric Food Chem*. 2015;63:1521–1531.

Cuendet M et al. *J Nat Prod*. 2006;69(3):460-463.
Furue M et al. *Nutrients.* 2017;9(3):E223.

Metagenics Institute

Depurinating Adducts—Major Metabolic Pathway in Cancer Initiation by Estrogens

Catechol estrogen-3,4-quinones react with DNA to form depurinating estrogen-DNA adducts

Loss of these adducts leaves apurinic sites in the DNA, generating mutations that can lead to the initiation of cancer

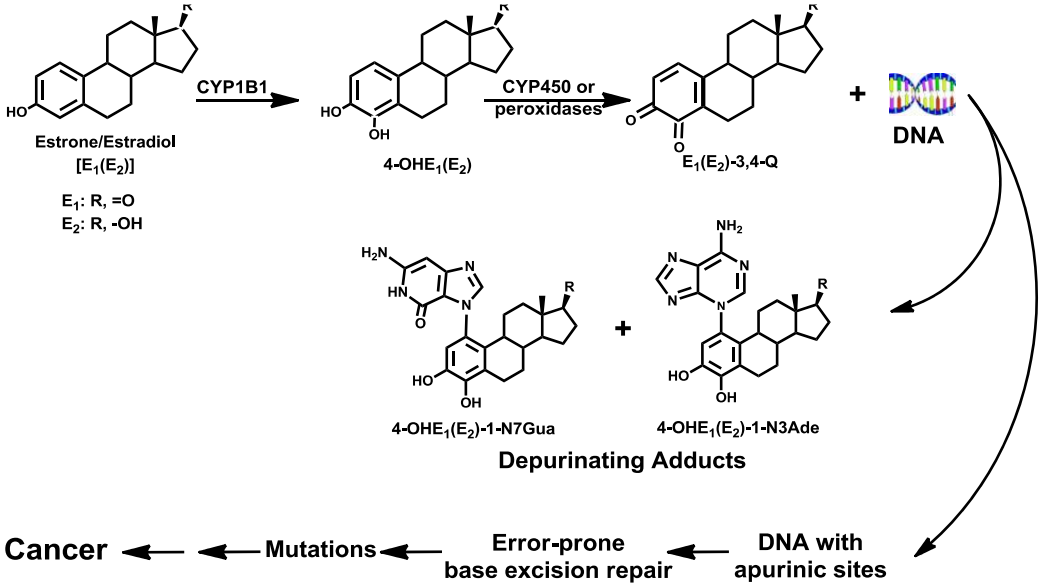


Figure: Cavalieri EL et al. *Clin Trans Med.* 2016;5:12. https://creativecommons.org/licenses/by/4.0/. Accessed February 25, 2019.

Metagenics Institute

Depurinating Estrogen-DNA Adducts and Disease Risk[1-3]

- **Women**
  - Higher levels of depurinating estrogen-DNA adducts in those diagnosed with breast cancer, and in those with higher risk for breast cancer
  - "Observation of high levels of depurinating estrogen-DNA adducts in high risk women before the presence of breast cancer indicates that adduct formation is a critical factor in breast cancer initiation."[3]
  - Higher levels in women with thyroid or ovarian cancer
- **Men**
  - Higher levels in men with prostate cancer or non-Hodgkin lymphoma
- Reducing the levels of estrogen-DNA adducts could prevent the initiation of human cancer

1. Cavalieri EL et al. *IUBMB Life.* 2012;64(2):169–179.
2. Cavalieri EL et al. *Clin Trans Med.* 2016;5:12.
3. Cavalieri EL et al. *Int J Cancer*. 2017;141(6):1078-1090.

Metagenics Institute

Altered Estrogen Homeostasis in Breast Cancer: E1 and E2 Levels Are Raised in Cancer vs. Controls

- Estrogen exposure has been linked to risk for breast cancer[1]
- Breast biopsy tissues from 49 women without breast cancer (controls) and 28 with breast cancer (cases) were analyzed for estrogen metabolites and catechol estrogen quinone-glutathione conjugates[2]
- **Results showed:**
  - Higher levels of E1 and E2 with more 2-catechol estrogen (CE) than 4-CE in controls vs. cancer cases
  - 4-CE were three times higher than 2-CE in cancer cases, and 4-CE was nearly four times higher vs. controls
  - Less O-methylation observed for the CE in cases; level of catechol estrogen quinone conjugates in cancer cases was three times that in controls, suggesting a higher probability for the quinones to react with DNA and generate mutations that may initiate cancer
  - Levels of 4-CE and quinone conjugates were highly significant predictors of breast cancer

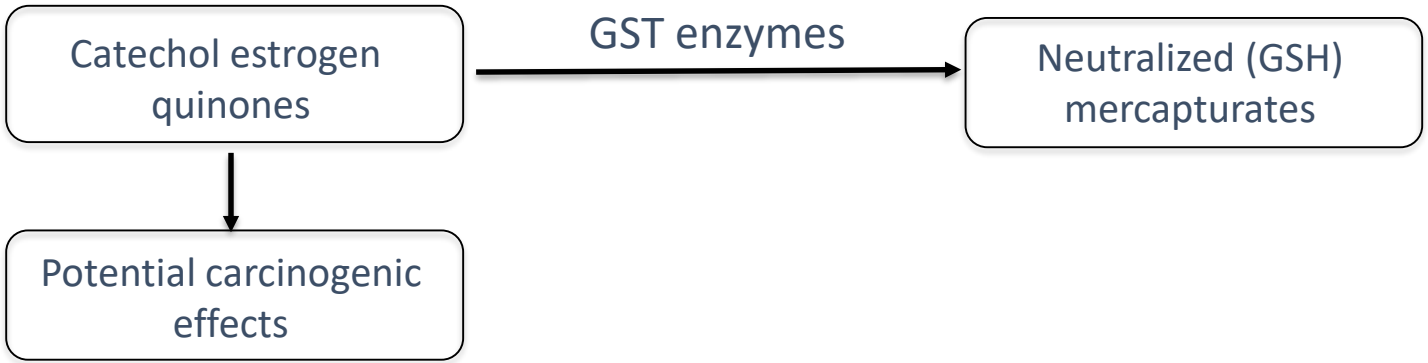
These results suggest that some catechol estrogen metabolites and conjugates could serve as biomarkers to predict risk of breast cancer

1. Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
2. Rogan EG et al. *Carcinogenesis*. 2003;24(4):697–702.

Metagenics Institute

Glutathione S-Transferases

Glutathione S-transferases (GSTs) include a complex of enzymes, whose main function is to attach a glutathione group to a biotransformed metabolite, reducing its carcinogenicity



Strange RC et al. *Mutat Res*. 2001;482(1-2):21-26.

Effect of Hormone Replacement Therapy (HRT) on Phase II Detox Pathways (COMT,* GST**)

COMT and/or GST genotypes plus HRT = increased risk for breast cancer

- COMT and GST single-nucleotide polymorphisms (SNPs) are associated with an increased risk of breast cancer
- This risk increases substantially with addition of HRT in affected patients
  - After initiating HRT, women with low activity SNP had increased rates of breast cancer
- Combined COMT and GST genotypes and hormone replacement therapy are associated elevated breast cancer risk

*COMT: catechol-O-methyltransferase

**GST: glutathione S-transferase

Mitrunen K et al. *Pharmacogenetics*. 2002;12(1):67-72.

Enhancing Reduced Glutathione (GSH) Status[1-2]

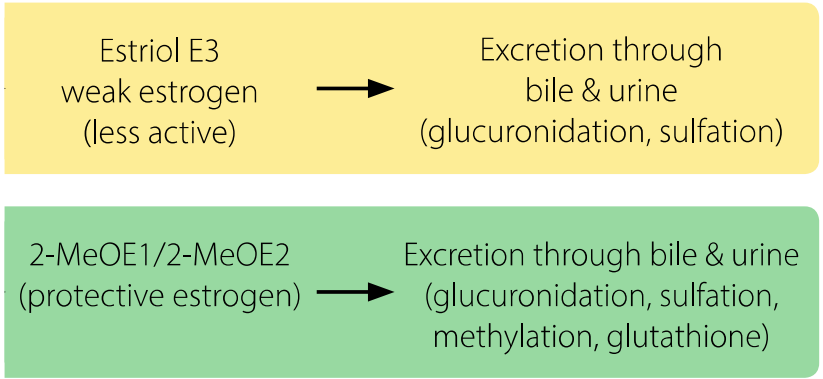
- Support for glutathione conjugation also involves enhancing reduced glutathione (GSH) status
- Glutathione is a low-molecular weight tripeptide containing residues of cysteine, glutamate, and glycine
- Most glutathione from foods and supplements is poorly absorbed
- Sulfur-containing amino acids methionine and cystine are important precursors to glutathione formation; their depletion leads to depressed GSH levels
- N-acetylcysteine (NAC) has also been used to restore depleted GSH levels in a clinical setting[2]

1. Wu G et al. *J Nutr*. 2004;134(3):489-492.
2. Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.

Metagenics Institute

Phase II UDP-Glucuronosyltransferases

- Function: Catalyze the covalent linkage of glucuronic acid from UDP-glucuronic acid to an accepting functional group on the molecule, a process referred to as glucuronidation
- Glucuronidation occurs primarily in the liver but can occur in other tissues, such as the small intestine
- Bilirubin, specifically, is principally conjugated by UGT1A1 in hepatocytes and then excreted with bile into the intestinal tract

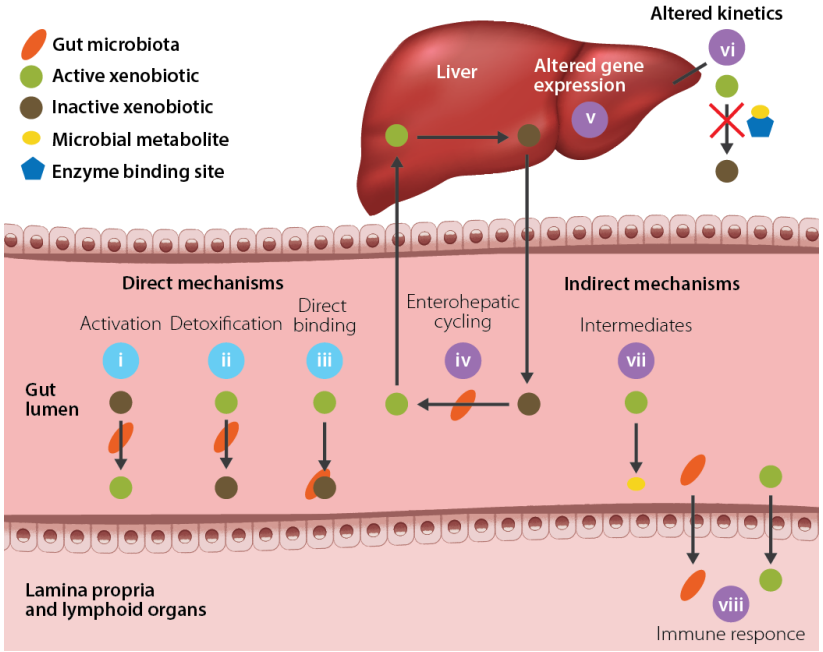


Bock KW. *Biochem Pharmacol*. 2015;96(2):77-82.
Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.

The Gut-Liver Connection

The microbiome is the “new liver”

- Recent studies have suggested the metabolic capacity and capabilities of gut microbiota to be similar to those of the liver.[1]
- Colonic bacteria produce β-glucuronidase (BG) enzymes, facilitating reabsorption of excreted estrogen metabolites.[2]



Adapted from: Carmody RN et al. *J Clin Invest.* 2014;124(10):4173-4181.

1. Carmody RN et al. *J Clin Invest.* 2014;124(10):4173-4181.
2. Hodges RE et al. *J Nutr Metab.* 2015;2015:760689.

Metagenics Institute

Promoting a Healthy Gut Microbiome Positively Impacts Estrogen Homeostasis

- A woman's lifetime burden of estrogen exposure may reflect (in part) the metabolic functioning of her estrobolome[1]
- Some gut bacteria may produce excess β-glucuronidase (BG)[2]
  - BG—enzymes that deconjugate glucuronic acid from estrogen
  - Excess BG is seen in intestinal dysbiosis
  - ↑ BG = ↓ excretion of estrogen (more recirculation)
  - ↑ recirculation = ↑ risk of reuptake and reabsorption of free estrogens
  - Results in greater risk for estrogen-driven cancers (breast, ovarian, and endometrial cancers)[3-4]

1. Baker JM et al. *Maturitas*. 2017;103:45-53.
2. Plotnikoff GA. *Glob Adv Health Med*. 2014;3(3):33-43.
3. Plottel CS et al. *Cell Host Microbe*. 2011;10(4):324–335.
4. Fernández MF et al. *Int J Environ Res Public Health*. 2018;15(8):E1747.

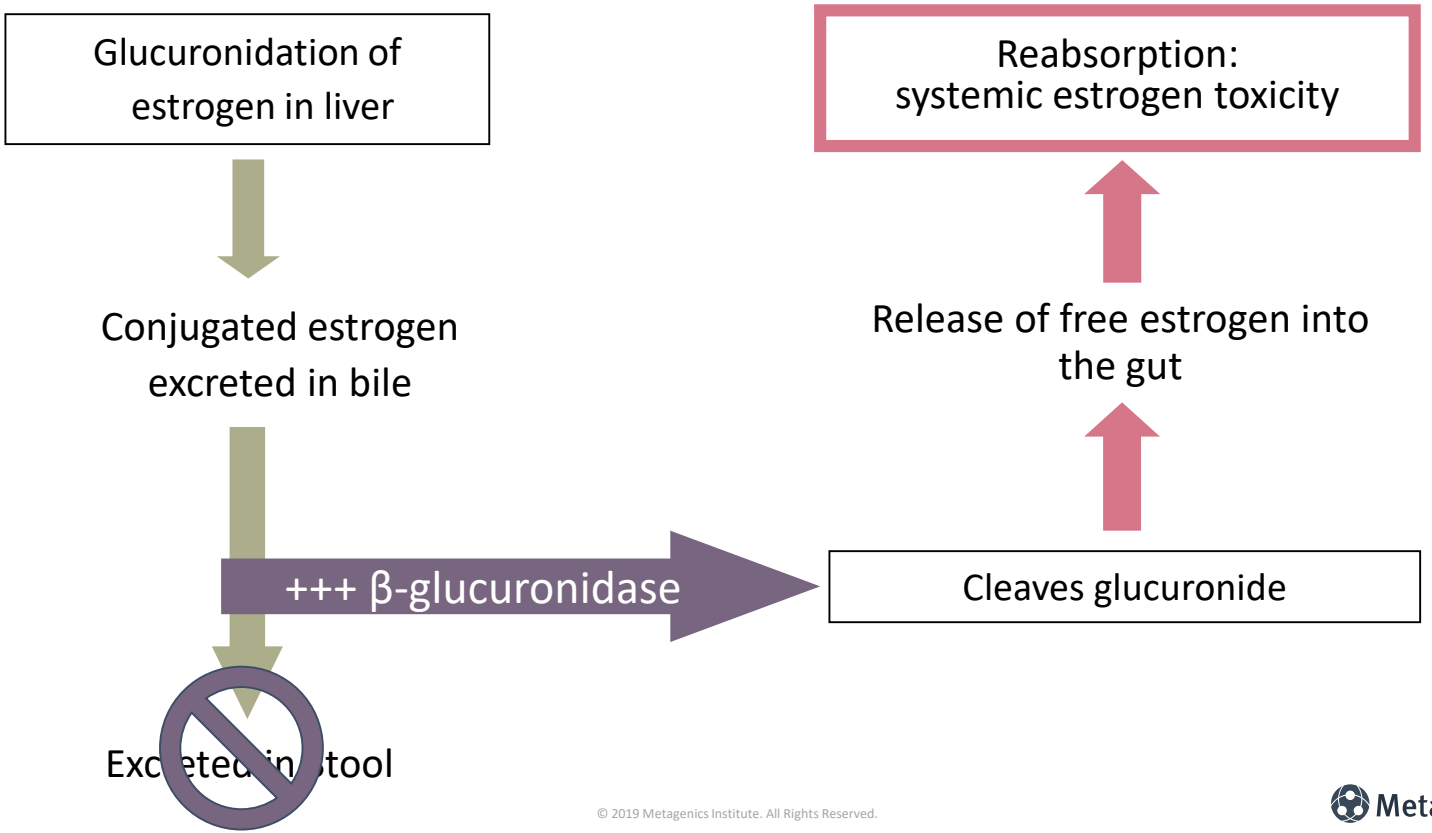
Interventions That Target the Estrobolome Also Affect Estrogens

- Interventions that modify the bacterial constituents of the estrobolome[1] could also modulate its functional activity to deconjugate estrogens
- Manipulations that specifically target species with β-glucuronidase and β-glucuronide activities could aid in reducing estrogen-related cancer risk
- Changing bacterial populations to diminish hydroxylation and reductive functions can be accomplished with use of antimicrobial agents, prebiotics, or probiotics
- Intestinal dysbiosis increases β-glucuronidase[2]
- Metabolites generated from commensal bacteria when the diet is rich in fat and red meat favor the growth of *E. coli, Klebsiella, Enterobacter,* and *Citrobacter,* leading to a dysbiotic state[3]
- *E. coli* is able to produce potent β-glucuronidases, deconjugating estrogens in the intestinal lumen and thus contributing to the higher estrogenic burden of the host

1. Plottel CS et al. *Cell Host Microbe*. 2011;10(4):324-335.
2. Baker JM et al. *Maturitas*. 2017;103:45-53.
3. Shapira I et al. *ISRN Oncol*. 2013;2013:693920.

Estrobolome Revisited

Phase II glucuronidation and estrogen excretion



Metagenics Institute

KEY TAKEAWAYS

Estrobolome influences estrogen burden through reabsorption of conjugated estrogens

Interventions target:

- Intestinal dysbiosis
- Efficient Phase 2 hepatic glucuronidation of estrogen

Supporting 3 Phases of Detoxification Reduces Estrogen Body Burden, Toxic E Metabolites, and Favors Protective Metabolite MeOHE

Endocrine-disrupting chemicals

- ❑ Organochlorine chemicals such as vinyl chlorides, dioxins, PCBs, and perchloroethylene (~half of "endocrine disrupters" are in this class)
- ❑ Aromatic hydrocarbons, phthalates, phenols, and some surfactants
- ❑ Medications—hormone replacement, oral contraceptives, tamoxifen, and cimetidine
- ❑ Hormones in animal products consumed by humans

Endogenous estrogens

- ❑ Estradiol (E2)
- ❑ Estrone (E1)
- ❑ Estriol (E3)
- ❑ Hydroxylated estrogen metabolites
- ❑ Methoxylated estrogen metabolites (MeOHE)
- ❑ Oxidized catechol estrogen-3,4-semiquinones/quinones
- ❑ Aromatization of androgens in peripheral tissues
- ❑ Estrobolome—reabsorbed estrogens

Diamanti-Kandarakis E et al. *Endocr Rev*. 2009;30(4):293-342.

KEY TAKEAWAYS

The body manages **excess estrogen as a toxin**

Phase I bioactivation produces reactive metabolites that are neutralized through **Phase II conjugation** and eliminated through **Phase III excretion**

In Phase I, estrogen can be activated into **2-OH (favorable), 16α-OH (less favorable), and 4-OH (unfavorable) metabolites**

Multiple Phase II systems prepare activated metabolites for excretion via **conjugation**

Methylation (COMT) and **reduction** of 2,4 catechol-estrogen quinones via the Nrf2 system is critical to protecting estrogen sensitive tissues from overstimulation

Phase I metabolites **induce Nrf2**, and Nrf2 induces the expression of endogenous antioxidants like superoxide dismutase **(SOD)** and Phase II enzymes like glutathione S-transferase **(GST)**

Nutritional Modulation of Estrogen Metabolism

Convergence of neuroendocrine science and nutritional bioactive research opens the door to a new understanding of ways to reduce risk of estrogen-sensitive illness

The fate of estrogen and its metabolites are closely linked to inflammation, insulin insensitivity and elevated stress hormones

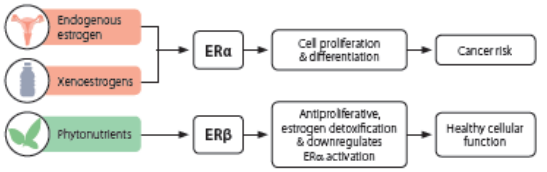
Estrogen metabolism and nutritional influences

Estrogen is the primary hormone responsible for sexual and reproductive development in women. Once puberty begins, the body uses estrogen to regulate the first half of the menstrual cycle and then metabolizes the hormone for elimination via urination and defecation. Dietary and lifestyle modifications that support a healthy weight, like consuming a nutrient-dense dietary pattern (e.g., increasing intake of fiber and phytoestrogens) and being physically active, have been linked to the modulation of estrogen metabolism. In addition, many nutrients and nutritional bioactives have been studied for their influence on pathways of estrogen metabolism and detoxification, including but not limited to isoflavones, indole-3-carbinol, B vitamins, magnesium, limonene, calcium D-glucarate, and antioxidants.

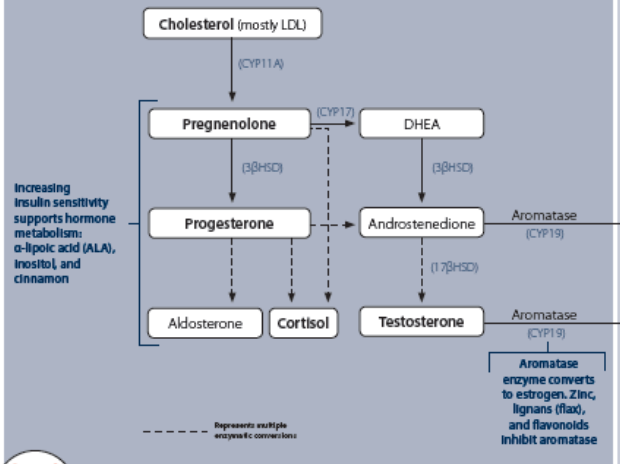
Estrogens & estrogen receptor sensitivity

Estrogen receptors (ER) are present in both men and women. Endogenous estrogens, environmental xenoestrogens, and their metabolites selectively bind to estrogen receptors. Various phytonutrients, such as phytoestrogens, may moderate their binding, modulating cell signaling to support hormone balance.

Phytoestrogen (plant-derived estrogens) examples include lignans, isoflavones (genistein, daidzein), and resveratrol. Vitamin B$_6$ helps modulate tissue response.

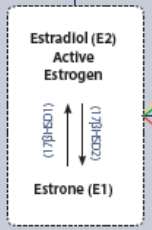


Production and conversion of estrogen

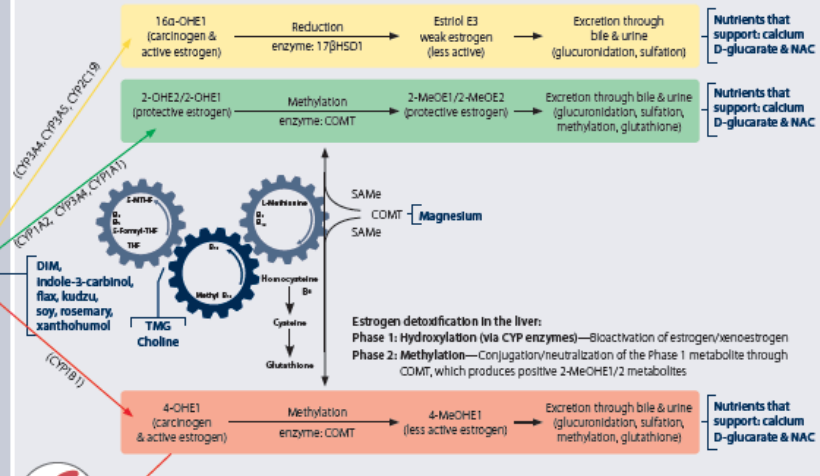


The body's systemic pool of estrogen

Lignans (found in fiber-rich foods) & isoflavones (found in legumes) help regulate production of sex hormone-binding globulin (SHBG), which assists in the regulation of free estrogens, testosterone, and dihydrotestosterone in circulation



Estrogen detoxification: bioactivation, conjugation, and elimination



Estrobolome—gut & estrogen connection

The gut plays a vital role in the body's ability to clear unwanted estrogen metabolites. Gut dysbiosis can contribute to β-glucuronidase activity, which allows estrogen to reenter circulation and increase the estrogen pool. Effects can be unbalanced hormones. Proper gut health, nutrition, and the types and amounts of macronutrients support estrogen biotransformation and clearance.

- **5R gut protocol, probiotics, prebiotics, fiber (apple pectin)**
- **β-glucuronidase inhibitors: probiotic strain *L. acidophilus* NCFM®, calcium D-glucarate; lignans**
- **Flax lignans support excretion**

Liver & estrogen detoxification

Balancing Phase I and Phase II enzyme systems in the liver supports healthy estrogen detoxification and encourages the clearance of genotoxic 4-OHE1 metabolites through the induction of Nrf2, quinone reductase (NQO1), GSTs,* and GSH.

KEY: **CLA:** conjugated linoleic acid; **COMT:** catechol-O-methyltransferase; **DHEA:** dehydroepiandrosterone; **5-Formyl-THF:** 5-formyltetrahydrofolate; **HRT:** hormone replacement therapy; **5-MTHF:** 5-methyltetrahydrofolate; **NAC:** N-acetylcysteine; **SAMe:** S-adenosylmethionine; **SAH:** S-adenosylhomocysteine; **SHBG:** sex hormone-binding globulin; **THF:** tetrahydrofolate; **TMG:** trimethylglycine; **Nrf2:** nuclear factor erythroid 2 (NF-E2) p45-related factor 2; **NQO1:** NAD(P)H:quinone oxidoreductase 1; **GST:** glutathione S-transferase; **GSH:** reduced glutathione; **2-OHE1:** 2-hydroxyestrone; **2-OHE2:** 2-hydroxyestradiol; **2-MeOE1:** 2-methoxyestrone; **2-MeOE2:** 2-methoxyestradiol; **4-OHE1:** 4-hydroxyestrone; **4-MeOHE1:** 4-methoxyestrone; **16α-OHE1:** 16α-hydroxyestrone

*GSTs: glutathione S-transferases are important enzymes of detoxification and intracellular binding proteins

Nutritional bioactives play key roles in estrogen metabolism

They share the same and different biochemical targets: production, storage/distribution, receptor binding/protection, Phase I and II detoxification

Metagenics Institute

Estrogen Metabolism	Nutrient Modulators
Production: Reduce estrogen body burden Support the microbiome and gut-estrogen connection (the estrobolome)	Lignans (flaxseed, spruce), flavonoids (chrysin), zinc, flavonoids (resveratrol, chrysin), isoflavones (genistein)[1-5]
	Calcium D-glucarate, fiber, lignans, prebiotics (guar gum), probiotics (*Lactobacillus, Bifidobacteria*)[6-9]
Systemic Estrogen Pool: Modulate circulating levels of estrogen	Fiber, lignans (flaxseed, spruce), isoflavones (soy, kudzu, red clover sources of genistein and daidzein)[10-14]
Receptor Sensitivity & Binding: Protect α and β estrogen receptors from triggers that stimulate expression of target genes within the cell affecting growth, health, and function of estrogen responsive tissues	Isoflavones (genistein, daidzein, puerarin, biochanin-A), lignans (flaxseed, spruce), indole-3-carbinol, resveratrol, vitamin B_6, rosemary, DIM, indole-3-carbinol, xanthohumol (from hops), curcumin (from turmeric)[15-21]
Detoxification—Biotransformation, conjugation, elimination: Promote healthy estrogen metabolite concentrations and ratios that influence estrogen sensitive tissues	Cruciferous vegetables, indole-3-carbinol, rosemary, isoflavones (soy, kudzu, clover) [22-27]
	Vitamins A, E, & C, N-acetylcysteine, curcumin (from turmeric) , green tea, lycopene, α-lipoic acid, flavonoids, superoxide dismutase (SOD) (from melon), xanthohumol (from hops)[28-32]
	Folate, vitamins B_2, B_6, & B_{12}, trimethylglycine, magnesium[33-35]
	Adequate dietary protein, healthy fats, adequate essential fatty acids, complex carbohydrates, curcumin (from turmeric), D-limonene, NAC, magnesium, vitamins B_2, B_6, & B_{12}, flavonoids[36-43]

Reference list located on slide 84

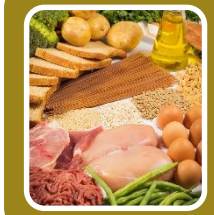
Estrogen Metabolism	Mechanisms	Nutrient Modulators
Production: Reduce estrogen body burden Support the microbiome and gut-estrogen connection (the estrobolome)	Inhibit activity of aromatase (CYP19), which converts androgens into estrogens	Lignans (flaxseed. spruce), flavonoids (chrysin), zinc, flavonoids (resveratrol, chrysin), isoflavones (genistein) [1-5]
	Inhibit activity of β-glucuronidase, which deconjugates estrogens in the large intestine, allowing them to be reabsorbed and re-metabolized. Promote the excretion conjugated estrogen glucuronides	Calcium D-glucarate, fiber, lignans, prebiotics (guar gum), probiotics (Lactobacillus, Bifidobacteria) [6-9]
Systemic Estrogen Pool: Modulate circulating levels of estrogen	Increase circulating concentrations of sex hormone binding globulin (SHBG), thus reducing levels of unbound, active estrogens	Fiber, lignans (flaxseed, spruce), isoflavones (soy, kudzu, red clover sources of genistein and daidzein) [10-14]
Receptor Sensitivity & Binding: Protect α and β estrogen receptors from triggers that stimulate expression of target genes within the cell affecting growth, health, and function of estrogen responsive tissues	Protect ERα and ERβ in hormonal and non-hormonal tissues (i.e. breast, uterus, ovaries, cervix, prostate, testes, bones, etc.) from over-exposure to xenoestrogens, excess endogenous estrogens (E1, E2) and catechol estrogen quinones	Isoflavones (genistein, daidzein, puerarin, biochanin-A) , lignans (flaxseed, spruce), indole-3-carbinol, resveratrol, vitamin B_6, rosemary, DIM, indole-3-carbinol, xanthohumol (hops derivative), curcumin (from turmeric) [15-21]
Detoxification—Biotransformation, conjugation, elimination: Promote healthy estrogen metabolite concentrations and ratios that influence estrogen sensitive tissues	Promote 2-OH (CYP1A2, CYP3A4, CYP1A1) hydroxylation over 4-OH (CYP1B1) and/or 16α-OH hydroxylation of estrogens	Cruciferous vegetables, indole-3-carbinol, rosemary, isoflavones (soy, kudzu, clover) [22-27]
	Reduce oxidation of catechol estrogens (2-OH and 4-OH) to catechol estrogen quinones and formation of depurinating DNA adducts by inducing Nrf2, quinone reductase (NQO1), glutathione S-transferase (GST), reduced glutathione	Vitamins A, E, & C, N-acetylcysteine, curcumin (from turmeric), green tea, lycopene, α-lipoic acid, flavonoids, superoxide dismutase (SOD) (from melon), xanthohumol (from hops)[28-32]
	Promote the methylation of catechol estrogens (2-OH and 4-OH) via COMT to form 4-MeOHE and 2-MeOHE	Folate, vitamins B_2, B_6, & B_{12}, trimethylglycine, magnesium[33-35]
	Promote the detoxification of estrogens by regulating Phase I and Phase II enzymes to produce neutralized (GSH) mercapturates, glucuronides, sulfates, reduced glutathione	Adequate dietary protein, healthy fats, adequate essential fatty acids, complex carbohydrates, curcumin (from turmeric), D-limonene, NAC magnesium, vitamins B_2, B_6, & B_{12}, flavonoids[36-43]

Reference list located on slide 84

References for slides 82 and 83

1. Huang M et al. *J Diabetes*. 2018;10(6):467-477.
2. Longcope C et al. *J Clin Endocrinol Metab*. 2000;85(1):293-296.
3. Balunas MJ et al. *Anticancer Agents Med Chem*. 2008;8(6):646–682.
4. Jeong HJ et al. *Arch Pharm Res.* 1999;22(3):309-312.
5. Oliveira GA et al. *J Toxicol Environ Health A*. 2012;75(16-17):1000-1011.
6. Maruti SS et al. *Cancer Epidemiol Biomarkers Prev*. 2008;17(7):1808–1812.
7. Chiu CY et al. *Mar Drugs.* 2017;15(7):234.
8. Kwa M et al. *J Natl Cancer Inst*. 2016;108(8):djw029.
9. Walaszek K et al. *Cancer Detect Prev*. 1997;21:178-190.
10. Kumar NG et al. *Cancer*. 2002;94(4):1166–1174.
11. Pino AM et al. *J Clin Endocrinol Metab*. 2000;85(8):2797-2800.
12. Nowak DA et al. *Curr Top Nutraceutical Res*. 2007;5(4):177–181.
13. Huang M et al. *J Diabetes*. 2018;10(6):467-477.
14. Longcope C et al. *J Clin Endocrinol Metab.* 2000;85(1):293-296.
15. Xu X et al. *Cancer Epidemiol Biomarkers Prev*. 2000;9(8):781-786.
16. Bowers JL et al. *Endocrinology*. 2000;141(10):3657-3667.
17. Chadwick LR et al. *Phytomedicine*. 2006;13(1-2):119-131.
18. Dalessandri KM et al. *Nutr Cancer.* 2004;50(2):161-167.
19. Firestone GL et al. *Mol Endocrinol*. 2009;23(12):1940–1947.
20. Caruso JA et al. *Cell Cycle*. 2014;13(16):2587–2599.
21. Goyal A et al. *J Food Sci Technol*. 2014;51(9):1633–1653.
22. Higdon JV et al. *Pharmacol Res.* 2007;55(3):224–236.
23. Rajoria S et al. *Thyroid.* 2011;21(3):299–304.
24. Wang S et al. *Chem Res Toxicol.* 2016;29(7):1142–1150.
25. Gonzalez-Vallinas M et al. *Electrophoresis*. 2014;35(11):1719-1727.
26. Fuhrman BJ et al. *Cancer Epidemiol Biomarkers Prev.* 2009;18(10):2751–2760.
27. Lecomte S et al. *Int J Mol Sci.* 2017;18(7):1381.
28. Bak MJ et al. *Semin Cancer Biol*. 2016;40-41:170–191.
29. Fuhrman BJ et al. *Nutr J*. 2013;12:25.
30. Muzandu K et al. *Jpn J Vet Res.* 2005;52(4):173-184.
31. Zahid M et al. *Free Radic Biol Med*. 2010;49(3):392–400.
32. Kumar S et al. *Scientific World Journal*. 2013;2013:162750.
33. Mense SM et al. *Carcinogenesis*. 2009;30(7):1202–1208.
34. Goodman JE et al. *Carcinogenesis.* 2001;22(10):1661-1665.
35. Lucock M. *Mol Genet Metab*. 2000;71(1-2):121-138.
36. Dahl WJ et al. *Br J Nutr*. 2012;108 Suppl 1:S3-10.
37. Huskisson E et al. *J Int Med Res*. 2007;35(3):277-289.
38. Zhu BT et al. *Cancer Res.* 1998;58:2269-2277.
39. Lucock M. *Mol Genet Metab*. 2000;71(1-2):121-138.
40. O'Shaughnessy A et al. *Gynecol Obstet Invest*. 2001;52(4):237.
41. Parazzini F et al. *Magnes Res*. 2017;30(1):1-7.
42. Kelsey NA et al. *Molecules.* 2010;15(11):7792–7814.
43. Levine CB et al. *BMC Vet Res.* 2017;13:388.

Metagenics Institute



Protein, Fats, Carbs

Micronutrient Profile

Phytochemical Complexity: Oxidant Balance

Macro- and Micronutrient Influences on Estrogen—Energy Sources That Drive Tissue Protection and Efficient Detoxification

Protein, Healthy Fats, Essential Fatty Acids, Complex Carbohydrates, Vitamins and Minerals

Dahl WJ et al. *Br J Nutr*. 2012;108 Suppl1:S3-10.
Croteau E et al. *J Alzheimers Dis*. 2018;64(2):551-561.
Yamada K et al. *Tohoku J Exp. Med.* 2018;244(2):119-122.

Metagenics Institute

Why Focus on Macro- and Micronutrients?

Decrease in ***plant-based food*** consumption has led to…

- Decrease in healthy fat balance—***omega-3 fatty*** acids decline and increase in CVD, altered mood/cognition, chronic disease[1]
- Loss of ***micronutrient density*** in foods means less vitamins, minerals, and phytochemicals, which can lead to impaired detoxification[2]
- Shift in ***acid-base balance*** to chronic acid load due to higher animal protein intake contributes to inflammation[1,2]
- Inverted ***sodium-potassium ratio*** due to added salt can contribute to hypertension[1]
- Dramatic drop in ***fiber*** content leads to increase in gut problems like colon cancer and systemic inflammation[3]
- Increased ***simple sugars*** leads to metabolic syndrome, diabetes, cognitive decline[1]

1. USDA and USDHHS. *Dietary Guidelines for Americans*, 2015-2020. 8th ed. Washington, DC. https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf. Accessed March 16, 2019.
2. Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.
3. Kunzmann AT et al. *Am J Clin Nutr*. 2015;102(4):881-890.

The Problem:

What do we need to know?

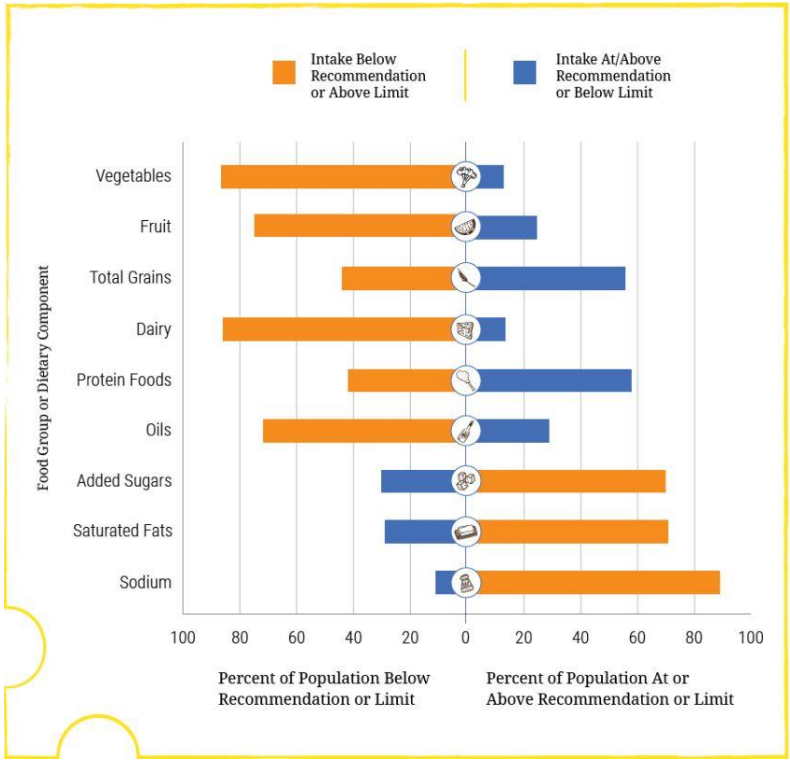
"About half of all American adults—117 million individuals—have one or more preventable, chronic diseases, many of which are related to poor quality eating patterns and physical inactivity."

USDA and USDHHS. *Dietary Guidelines for Americans*, 2015-2020. 8th ed. Washington, DC. https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf. Accessed March 16, 2019.

Metagenics Institute

Fruit & Vegetable Shortfalls Continue to Be Substantial

According to the CDC, only “12.2% (of Americans) met fruit intake recommendations and 9.3% met vegetable intake recommendations during 2015.”[2]



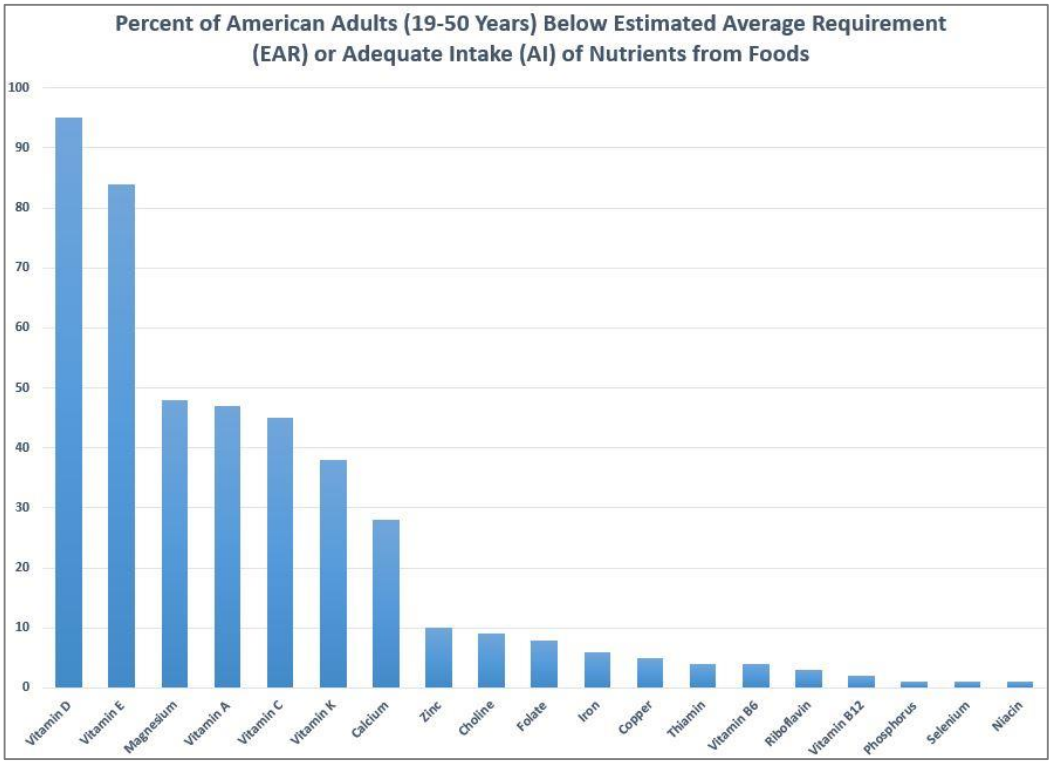
Source: What We Eat in America, NHANES 2007-2010.[1]

1. USDA and USDHHS. *Dietary Guidelines for Americans*, 2015-2020. 8th ed. Washington, DC. health.gov/dietaryguidelines/2015/guidelines/chapter-2/current-eating-patterns-in-the-united-states/. Accessed March 7, 2019.
2. Lee-Kwan SH et al. *MMWR Morb Mortal Wkly Rep.* 2017;66(45):1241–1247.

Metagenics Institute

Micronutrients of Concern

The best estimate for an individual's unobservable requirement is a Dietary Reference Intake (DRI) known as the Estimated Average Requirement (EAR), defined as the median requirement (i.e., meets ~50% of population's needs) of a nutrient for a given life stage and gender group.



Adapted from: Blumberg JB et al. *Nutrients.* 2017;9(12):E1325.

Metagenics Institute

Macronutrients Support Each Step in the Metabolism of Estrogens

Complex carbohydrates

Complex carbohydrates **attenuate glycemic** and **insulinemic responses**.[1]

Protein

Inadequate dietary protein may lead to decreases in overall cytochrome P450 activity, including cytochrome P450 1A2 (CYP1A2), which detoxifies estradiol. Amino acids of various types (e.g., taurine, glycine), whether endogenous or exogenous (from dietary sources) in origin, can be utilized for attaching to molecules for their excretion. For the benefits of providing a substrate to these enzymes, it is generally thought that dietary protein is required for an **effective detoxification** protocol.[2]

Fats

The types and amounts of dietary fats may play a role in determining balance among estrogens in the body. For instance, **high-fat diets may promote C-16α hydroxylation over C-2 hydroxylation**.[3]

1. Brewer RA et al. *Nutr Healthy Aging.* 2016;4(1):31-46.
2. Dahl WJ et al. *Br J Nutr*. 2012;108 Suppl 1:S3-10.
3. Musey PI et al. *J Clin Endocrinol Metab*. 1987;65(4):792-795.

Low Dietary Plant Protein and PMS-Related Athletic Impairment

- 135 female athletes aged 18-23 years divided into two groups: those without PMS-related impairment of athletic performance (n = 117) and those with PMS-related performance impairment (n = 18).
- Athletes whose performance was affected by PMS reported higher intake of animal protein than athletes whose performance was unaffected by PMS.
- Plant protein intake was lower among athletes with PMS-related impairment than among athletes without impairment.
- The proportion of dietary plant protein was lower among athletes with PMS-related impairment than those without impairment.

Yamada K et al. *Tohoku J Exp Med*. 2018;244(2):119-122.

Metagenics Institute

Monounsaturated Fats (MUFAs), Inflammation, and the Heart

- **Medium-chain triglycerides (MCT)** are triglycerides containing saturated fatty acids with lengths of 6-10 carbons
- Recent research suggests that consuming MCTs may support brain energy metabolism by increasing plasma ketone levels and brain ketone consumption.[1]
- **High-oleic** sunflower oil—sunflower oil with MUFA levels of 80% and above MUFAs, as oleic acid, improve lipid profile, maintain a balance of body weight and prevent palmitate-induced mitochondrial dysfunction, insulin resistance, and inflammatory signaling in neuronal cells and skeletal muscle.[2]

1. Croteau E et al. *J Alzheimers Dis*. 2018;64(2):551-561.
2. Perdomo L et al. *Cardiovas Diabet.* 2015;14:75.

Complex Carbohydrates Support Glucose Balance

It's all about timing:

Simple carbohydrates are absorbed quickly, causing blood sugar to rise and fall quickly (i.e., table sugar, honey, dairy products, fruits, fruit juice.)

Complex carbohydrates are starches that provide sustained release of sugar (i.e., whole grains, veggies)

Why eat complex:

Whole-grain nutrients: essential fatty acids, B vitamins, folate, vitamin E, zinc, magnesium, and fiber.

Fruit and starchy vegetables: contain many vitamins and minerals, as well as phytonutrients. Phytonutrients are compounds that form a plant's immune system. They help prevent disease in humans when eaten. There are thousands of phytonutrients, including major groups like carotenoids, flavonoids, lignans, curcuminoids, etc.

Harvard Health Publishing. https://www.health.harvard.edu/diet-and-weight-loss/carbohydrates--good-or-bad-for-you. Accessed February 27, 2019.
Gupta C et al. *J Complement Integr Med.* 2014;11(3):151-169.
Cummings JH et al. *Eur J Clin Nutr*. 2007;61 Suppl 1:S5-18.

Micronutrients and Amino Acids Support Healthy Methylation and Breast Protective 2- and 4-MeOH

The detoxification and excretion of the catechol estrogens occurs via Phase II methylation by the catechol-O-methyltransferase (COMT) enzyme uses SAMe as its methyl donor.

The important cofactors for enzymes are involved in estrogen conjugation and methylation.

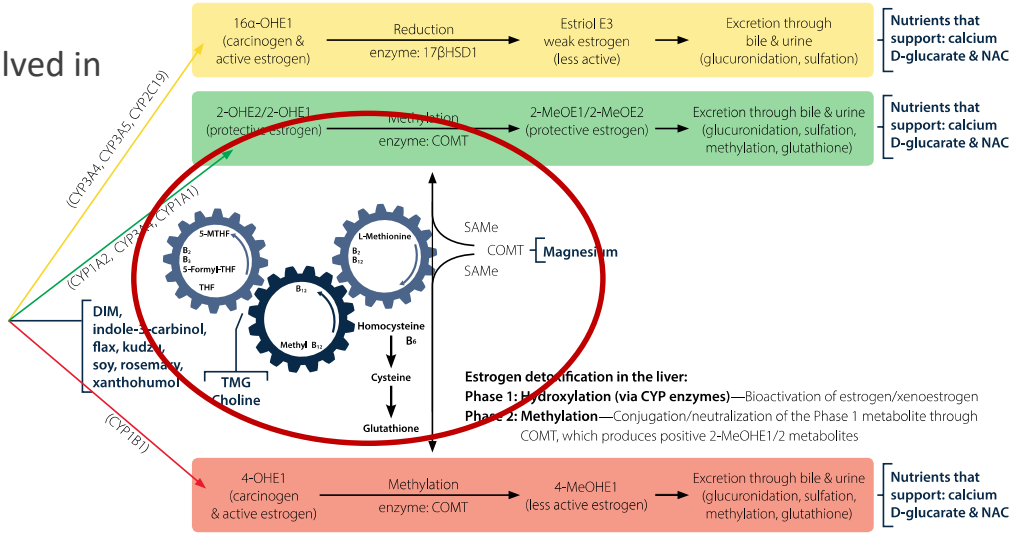
Magnesium

Vitamins B_2, B_6, and B_{12} (B_{12} as methylcobalamin) to promote healthy homocysteine levels

Folic acid (calcium L-5-methyltetrahydrofolate, a body-ready folate)

TMG (betaine)

Methionine



Huskisson E et al. *J Int Med Res*. 2007;35(3):277-289.
Zhu BT et al. *Cancer Res.* 1998;58:2269-2277.
Lucock M. *Mol Genet Metab.* 2000;71(1-2):121-138.

Metagenics Institute

Significance of Zinc in the Diet

Detoxification

- Zinc beneficially influences the activity and genetic expression of metallothionein, which is associated with the body's natural detoxification processes.[1]

Healthy testosterone levels

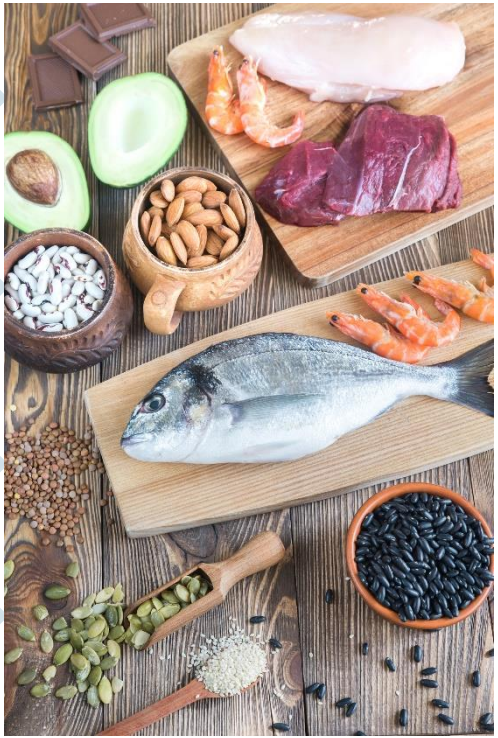
- Zinc deficiency reduces circulating luteinizing hormone and aromatization of testosterone, alters hepatic steroid metabolism, and modifies sex steroid hormone receptor levels.[2-3]

Prostate health

- Zinc is markedly decreased ~60–80% in prostate cancer compared to normal and benign prostate.[4-5]

Male function

- Positive correlation of zinc with sperm count, concentration, motility, and plasma testosterone concentration.[6]



1. Ruttkay-Nedecky B et al. *Int J Mol Sci.* 2013;14(3):6044-6066.
2. Prasad AS et al. *Nutrition.* 1996;12(5):344-348.
3. Om AS et al. *J Nutr.* 1996;126(4):842-848.
4. Costello LC et al. *Arch Biochem Biophys.* 2016;611:100–112.
5. Epstein MM et al. *Am J Clin Nutr.* 2011;93(3):586–593.
6. Murarka S et al. *Austin J Reprod Med Infertil.* 2015;2(2):1009.

Metagenics Institute

Magnesium Plays a Critical Role in Detoxification of Estrogens

- Magnesium promotes the methylation and excretion of catechol estrogens (2-OH and 4-OH) by COMT enzyme.[1]
- Magnesium also promotes estrogen detoxification by directly increasing the activity of glucuronyl transferase, an enzyme involved in hepatic glucuronidation.[1]
- Serum magnesium levels are lower while estrogen is elevated during the menstrual cycle.[2] Magnesium supplementation is effective in the prevention or amelioration of dysmenorrhea, premenstrual syndrome, menstrual migraine, and climacteric symptoms.[3]
- Magnesium is an essential element required as a cofactor for over 300 enzymatic reactions, and emerging evidence suggests that nearly 2/3 of the population in the Western world is not achieving the recommended daily requirement.[4]

1. Bolton JL et al. *Chem Res Toxicol.* 1998;11(10):1113-1127.
2. O'Shaughnessy A et al. *Gynecol Obstet Invest*. 2001;52(4):237-242.
3. Parazzini F et al. *Magnes Res*. 2017;30(1):1-7.
4. Schwalfenberg GK et al. *Scientifica (Cairo).* 2017;2017:4179326.

Metagenics Institute

Mixed Carotenoids and B Vitamin Supplementation Associated with Reduced Breast Cancer Risk

- **Prospective cohort studies—**
- α-carotene and β-carotene, lutein, zeaxanthin, and lycopene showed a protective effect on ER−/PR+ or ER−/PR− breast cancer.[1,2]

- **Large prospective cohort study** of 30,000 women showed a protective effect of B vitamin intake (dietary, supplemental, total) and breast cancer risk in middle-aged women.[3]

1. Bakker MF et al. *Am J Clin Nutr.* 2016;103(2):454–464.
2. Bae JM. *Epidemiol Health.* 2016;38:e2016024.
3. Egnell M et al. *Nutrients.* 2017;9(5):488.

Metagenics Institute

KEY TAKEAWAYS

Most people do not eat whole, fresh foods every day—fruits and veggies, legumes, seeds and nuts, whole grains, and quality proteins and healthy fats shown to decrease risk for chronic diseases.

Along with efforts to increase nutrient density of the diet, targeted nutritional supplementation with bioactives that mediate healthy estrogen metabolism supports at-risk hormonal and nonhormonal body systems—breast, immune, brain, bone, reproductive organs.

Multitarget, Multisystem Dietary Fibers and Lignans

- Insoluble and soluble dietary fibers
- Lignans: polyphenolic phytoestrogen bioactives from plants

Soluble vs. Insoluble Fiber

- Fiber consists of a variety of carbohydrates from plants that cannot be digested by the human small intestine
- Soluble fibers can dissolve in water, and are broken down by bacteria in the large intestine
  - Found in many foods: fruits, oats, vegetables, and legumes
- Insoluble fiber cannot dissolve in water, and therefore passes through the GI tract mostly intact
  - Found in many foods: wheat bran, whole grain foods, vegetables, nuts, and seeds

FDA. www.accessdata.fda.gov/scripts/InteractiveNutritionFactsLabel/factsheets/Dietary_Fiber.pdf. Accessed March 13, 2019.

Dietary Fiber Modulates Estrogen Metabolism

- Dietary fiber reduces the enterohepatic circulation of estrogens, thus promoting their excretion and making them less available for reabsorption[1,2]
- Dietary fibers:
  - Can **bind unconjugated estrogens** in the digestive tract, which are then excreted in the feces
  - **Beneficially affect the composition of intestinal bacteria and reduce intestinal β-glucuronidase activity,** resulting in a lowered deconjugation of estrogen and reduced reabsorption[3]
  - Also associated with **increased serum concentrations of SHBG**, which reduces levels of free estradiol[4]

1. Rock CL et al. *J Clin Oncol*. 2004;22(12):2379-2387.
2. Gaskins AJ et al. *Am J Clin Nutr*. 2009;90(4):1061–1069.
3. Lampe JW et al. *J Nutr*. 2002;132(6):1341-1344.
4. Huang M et al. *J Diabetes*. 2018;10(6):467-477.

Health Benefits of a Specific, Well-Researched Soluble Fiber: Partially Hydrolyzed Guar Gum (PHGG)

- PHGG is a soluble, fermentable fiber that is more palatable and less likely to cause adverse effects than whole guar gum.[1]
- In a clinical study in patients with chronic constipation, PHGG sped up colonic transit time and improved constipation symptoms.[2]
- It has prebiotic effects and helps support the growth of beneficial gut flora.[3]
- In a separate study, PHGG reduced bloating in a group of patients with irritable bowel syndrome (IBS).[4]
- It positively impacts glucose and lipid metabolism.[5]

1. McRorie JW Jr et al. *J Acad Nutr Diet.* 2017;117(2):251-264.
2. Polymeros D et al. *Dig Dis Sci.* 2014;59(9):2207–2214.
3. Russo L et al. *Saudi J Gastroenterol.* 2015;21(2):104–110.
4. Niv E et al. *Nutr Metab (Lond).* 2016;13:10.
5. Yoon SJ et al. *J Clin Biochem Nutr.* 2008;42:1-7.

Metagenics Institute

Lignans

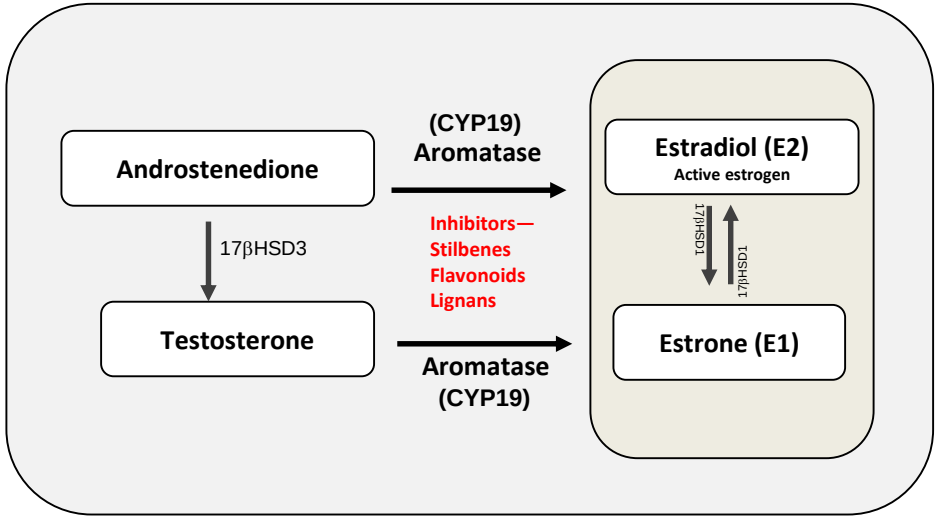
- Lignans are polyphenols that are found in many plant foods, including whole grains, seeds, fruit, vegetables, and legumes[1]
- Lignans give rise to metabolites called enterolignans—enterodiol, and enterolactone in the colon[2-3]
- Lignans are bioactive compounds exhibiting various biological properties, including antioxidant, anti-inflammatory and antitumor activity[4]

1. Adlercreutz H et al. *Crit Rev Clin Lab Sci.* 2007;44(5-6):483-525.
2. Landete JM. *Food Res Int.* 2012;46:410–424.
3. Durazzo A et al. *Molecules*. 2018;23(12):3251.
4. Ionkova I. *Mini Rev Med Chem*. 2011;11(10):843–856.

Managing Aromatase Can Be as Critical as Managing Estrogen

Lignans are weak phytoestrogens that can lower aromatase by:[1-6]

- Decreasing aromatase gene expression
- Inhibiting the aromatase enzyme (CYP19)
- In some cases, acting at both levels of regulation



1. Ma CX et al. *Nat Rev Cancer*. 2015;15(5):261-275.
2. Lephart ED. *Enzyme Res*. 2015;2015:594656.
3. Monteiro R et al. *J Steroid Biochem Mol Biol.* 2007;105(1-5):124–130.
4. Monteiro R et al. *J Agric Food Chem*. 2006;54(10):3535-3540.
5. Campbell DR et al. *J Steroid Biochem Mol Biol.* 1993;46(3):381-388.
6. Brooks JD et al. *J Steroid Biochem Mol Biol*. 2005;94(5):461-467.

Metagenics Institute

Specific, Well-Researched Lignan with Relevance to Estrogen:
7-Hydroxymatairesinol (HMR) lignans modulate aromatase activity

- The lignan 7-hydroxymatairesinol (HMR) is a naturally occurring plant lignan from the Norway spruce (*Picea abies*) and precursor of the mammalian lignan enterolactone (EL)[1]
- HMR lignans increase production of enterolactone, which has been associated with reduced aromatase activity[1]
- HMR lignans act like estrogen; stimulates the synthesis and the circulating levels of SHBG[1]
- Supplementation with a mixture of indole-3-carbinol and HMR lignan in pre- and postmenopausal women significantly increased estrogen C-2 hydroxylation and 2:16α-OHE ratio[2]
- In a clinical study, treatment with 7-HMR significantly improved menopausal symptoms in postmenopausal women[3]

1. Jacobs DR et al. *Br J Nutr.* 2002;88(2):111–116.
2. Laidlaw M et al. *Breast Cancer (Auckl).* 2010;4:85-95.
3. Udani JK et al. *J Am Coll Nutr*. 2013;32(6):428-435.

Metagenics Institute

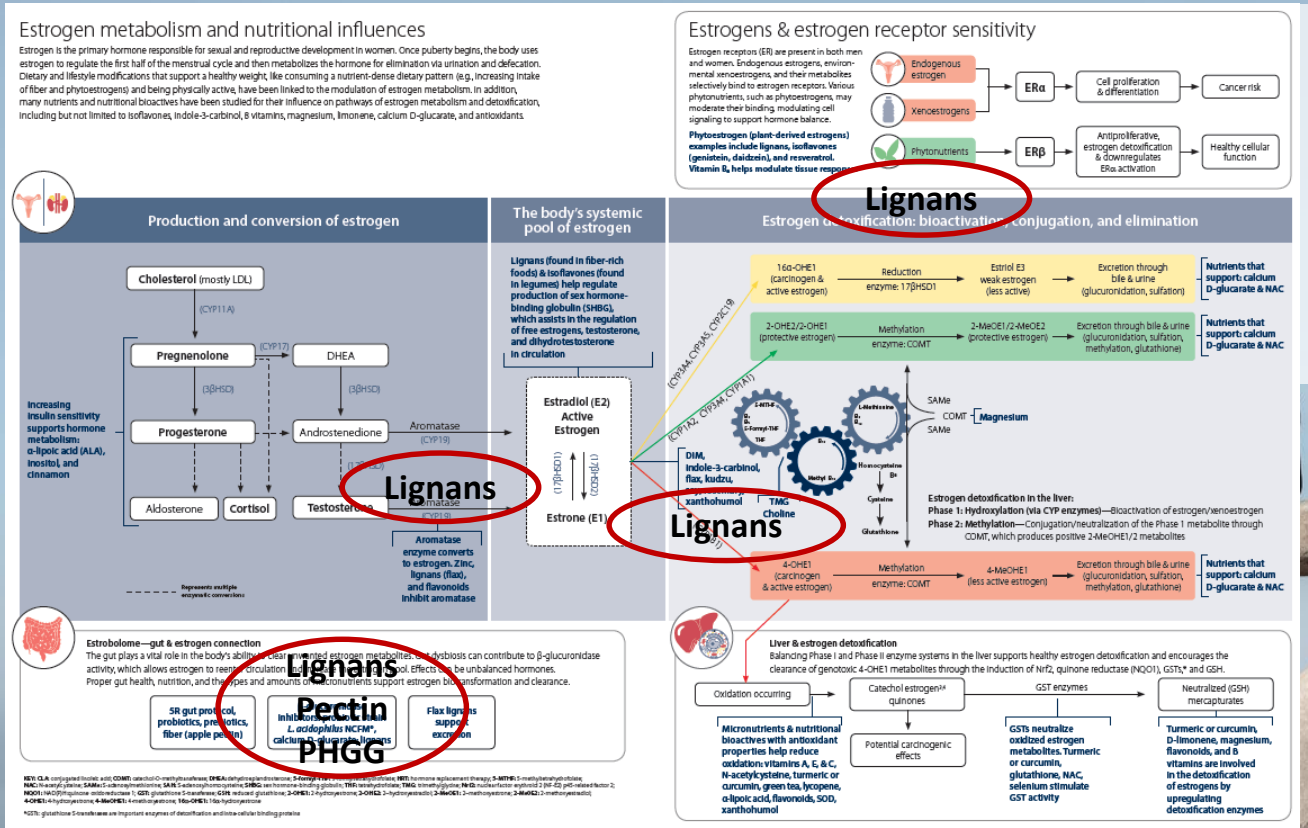
Specific, Well-Researched Lignan with Relevance to Estrogen:
Secoisolariciresinol diglucoside (SDG) lignan from flaxseed hull

- The major active bioactive of flaxseed hull lignan is secoisolariciresinol diglucoside (SDG)
- SDG flaxseed hull lignan is metabolized by intestinal bacteria to enterolignans—enterolactone and enterodiol
- The enterolignans displays weak estrogenic activity
- Clinical evidence
  - In clinical studies, supplementation with crushed and/or milled flaxseed improved the bioavailability of the enterolignans,[1] increased the excretion of enterodiol and enterolactone,[2-3] increased urinary 2-hydroxyestrogen (2-OH) excretion and urinary 2-hydroxyestrogen:16a-hydroxyestrone (2:16a-OH).[4-6]

1. Kuijsten A et al. *J Nutr.* 2005;135(12):2812–2816.
2. Hutchins AM et al. *Cancer Epidemiol Biomarkers Prev.* 2000;9(10):1113–1118.
3. Tarpila S et al. *Eur J Clin Nutr.* 2002;56(2):157-165.
4. Brooks JD et al. *Am J Clin Nutr.* 2004;79(2):318–325.
5. Haggans CJ et al. *Cancer Epid Bio & Prev.* 2000;9(7):719–725.
6. Sturgeon SR et al. *Nutr Cancer*. 2010;62(2):175-180.

KEY TAKEAWAYS

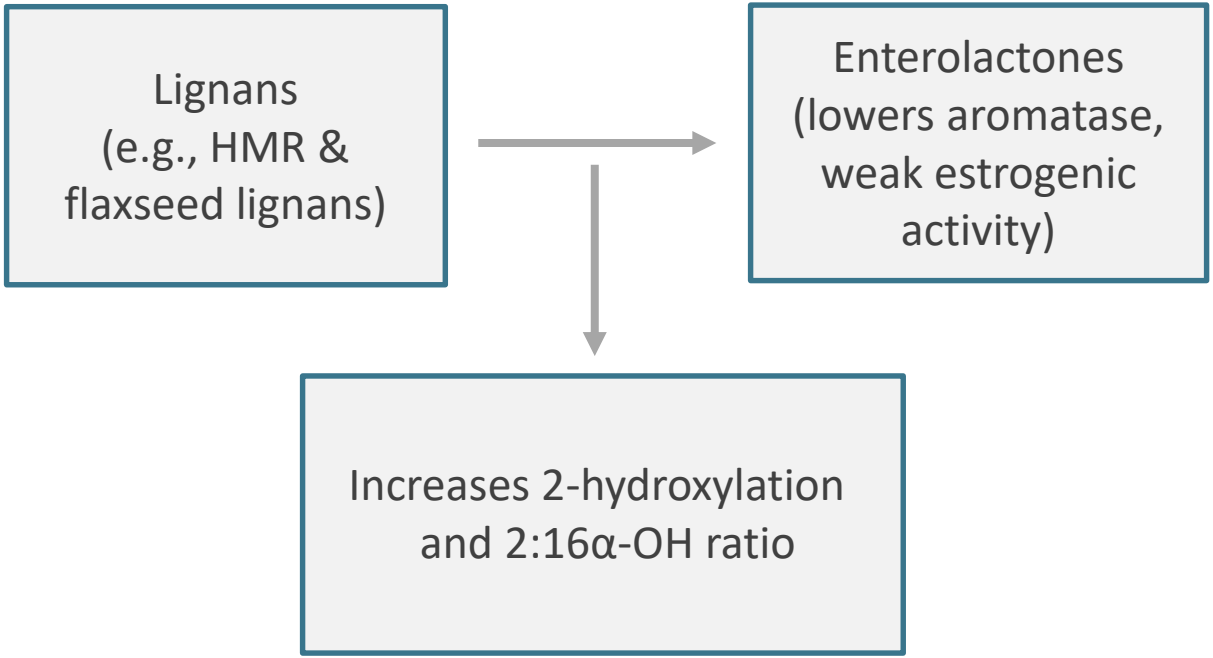
Lignans, soluble fibers, insoluble fibers and estrogen metabolism



- Inhibit activity of aromatase (CYP19)
- Increase circulating SHBG
- Inhibit activity of β-glucuronidase
- Promote the excretion conjugated estrogen glucuronides
- Protect ERα and ERβ in hormonal and nonhormonal tissues

KEY TAKEAWAYS

Benefits of Lignans on Estrogen Metabolism



Multitarget, Multisystem Cruciferous Vegetables

- Key Nutritional Bioactives from Crucifers:
  - Glucosinolates
  - DIM (3,3’-diindolylmethane)
  - Indole-3-carbinol
  - Sulforaphane

Multitarget Nutrients

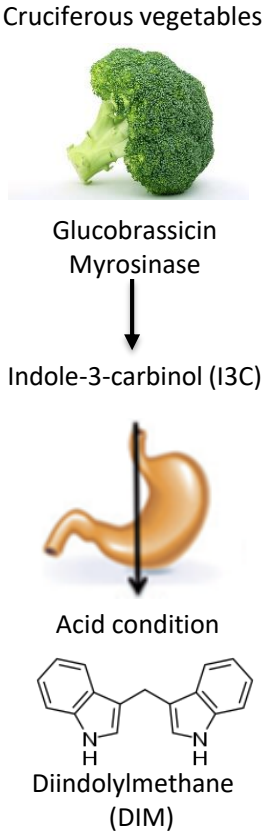
Health Benefits of Cruciferous Vegetables

- Consumption of cruciferous vegetables has been associated with a decreased risk of several chronic diseases including cancers
- Cruciferous vegetables accumulate significant concentrations of glucosinolates, which are a group sulfur and nitrogen containing compounds that contribute to the bitter taste of these vegetables
- Glucosinolates are responsible for many positive health benefits associated with cruciferous consumption

Boddupalli S et al. *Front Genet*. 2012;3:7.
Thomson CA et al. *Nutr Rev.* 2016;74(7):432–443.

Glucosinolates and Their Breakdown Products Are Absorbed or Metabolized in the Gut

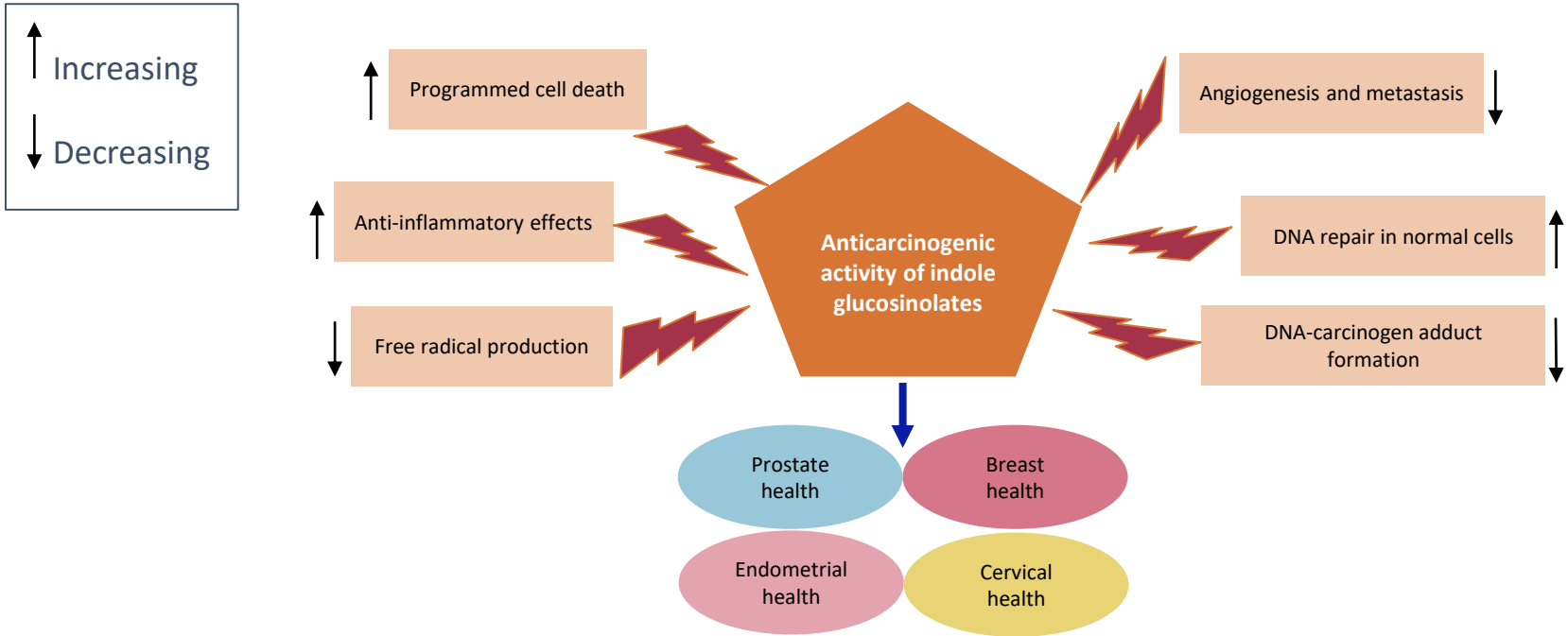
- Glucobrassicin is the most abundant glucosinolate and converted *in vivo* to unstable indoles, mainly indole-3-carbinol. This conversion requires myrosinase enzyme.
- I3C is an unstable compound in acidic conditions and undergoes rapid oligomerization to form a mixture of acid condensation products, with 3,3'-diindolylmethane (DIM) being the predominant compound.
- Glucoraphanin is another important glucosinolate, which myrosinase can convert to sulforaphane



Barba FJ et al. *Front Nutr.* 2016;3:24.
Thomson CA et al. *Nutr Rev.* 2016;74(7):432–443.
Kassie F et al. *Cancer Res*. 2007;67(13):6502–6511.
Guerrero-Beltrán CE et al. *Exp Toxicol Pathol.* 2012;64(5):503–508.

Metagenics Institute

Protective Effects of Indole Glucosinolates (I3C and DIM) and Mode of Action



Adapted from: Maruthanila VL et al. *Adv Pharmacol Sci.* 2014;2014:832161.

Metagenics Institute

Clinical Studies on DIM Have Utilized Absorption-Enhanced DIM Instead of Insoluble Active DIM

- Pharmacokinetic models have indicated that crystalline DIM may be poorly absorbed in the body.[1]
- All the clinical trials have only used an absorption-enhanced DIM formulation, including clinical trials using microencapsulated absorption-enhanced DIM to study HPV,[1] cervical dysplasia,[2] uterine,[3] and prostate health.[4]
- Absorption-enhanced DIM is the only DIM in clinical trials sponsored by the National Cancer Institute.
- Absorption-enhanced DIM has been studied in various applications surrounding estrogen metabolism and hormonal balance.[5-7]

1. Anderton MJ et al. *Drug Metab Dispos.* 2004;32(6):632-638.
2. Zeligs MA et al. *J Am Nutraceut Assoc*. 2005;8(1):5-15.
3. Zeligs MA et al. *Proc Am Assoc Cancer Res*. 2003;44:1268.
4. Zeligs MA et al. *Proc Am Assoc Cancer Res*. 2002;43:3198.
5. Teas J et al. *Cancer Detect Prev*. 2005;29(6):494-500.
6. Dalessandri KM et al. *Nutr Cancer*. 2004;50(2):161-167.
7. Bradlow HL et al. *J Endocrinol*. 1996;150 Suppl:S259-S265.

Metagenics Institute

Clinical Evidence for I3C and DIM

	I3C	DIM
Source	Formed as a hydrolysis product of glucobrassicin (a glucosinolate compound found in cruciferous vegetables)	Formed from I3C as an acid condensation product in an acidic environment (e.g., stomach)
Dose exerting efficacy based on clinical trials	300-400 mg per day[1-6]	(Absorption-enhanced DIM) 100-300 mg per day[7-9]
Activity	Converts into DIM, which exerts biological activity	Exerts biological activity
Clinical health benefits	Modulation of biotransformation and estrogen metabolism (e.g., a shift in metabolic pathway toward 2-OH from the 16α-OH) and other potentially chemopreventive activities	
Safety	• Well tolerated at up to 400 mg twice daily for 8 weeks[10] • Long-term effects of I3C on cancer risk in humans are not known	• Absorption-enhanced DIM well-tolerated at 300 mg daily for 12 months in women taking tamoxifen, with some discolored urine reported[9] • Long-term effects of absorption-enhanced DIM on cancer risk in humans are not known

1. Bradlow HL et al. *Cancer Epidemiol Biomarkers Prev*. 1994;3(7):591-595.
2. McAlindon TE et al. *Lupus*. 2001;10(11):779-783.
3. Michnovicz JJ et al. *J Natl Cancer Inst*. 1997;89(10):718-723.
4. Michnovicz JJ. *Int J Obes Relat Metab Disord*. 1998;22(3):227-229.
5. Wong GY et al. *J Cell Biochem Suppl*. 1997;28-29:111-116.
6. Reed GA et al. *Cancer Epidemiol Biomarkers Prev*. 2005;14(8):1953-1960.
7. Dalessandri KM et al. *Nutr Cancer*. 2004;50(2):161-167.
8. Rajoria S et al. *Thyroid*. 2011;21(3):299-304.
9. Thomson CA et al. *Breast Cancer Res Treat*. 2017;165(1):97-107.
10. Reed GA et al. *Cancer Epidemiol Biomarkers Prev*. 2006;15(12):2477-2481.

Metagenics Institute

Emerging Science—Select Human Studies with DIM

Breast studies

- ✓ Increased the ratio of 2-hydroxyestrones to 16α-hydroxyestrone and circulating levels of SHBG in breast cancer patients[1,2]
- ✓ Effective in cervical intraepithelial neoplasia treatment[3]
- ✓ Improved recurrent breast pain and soreness in premenopausal women[4]
- ✓ Well tolerated in breast cancer patients[5]
- ✓ Increased BRCA1 mRNA expression in women with a BRCA1 mutation[6]

Prostate studies

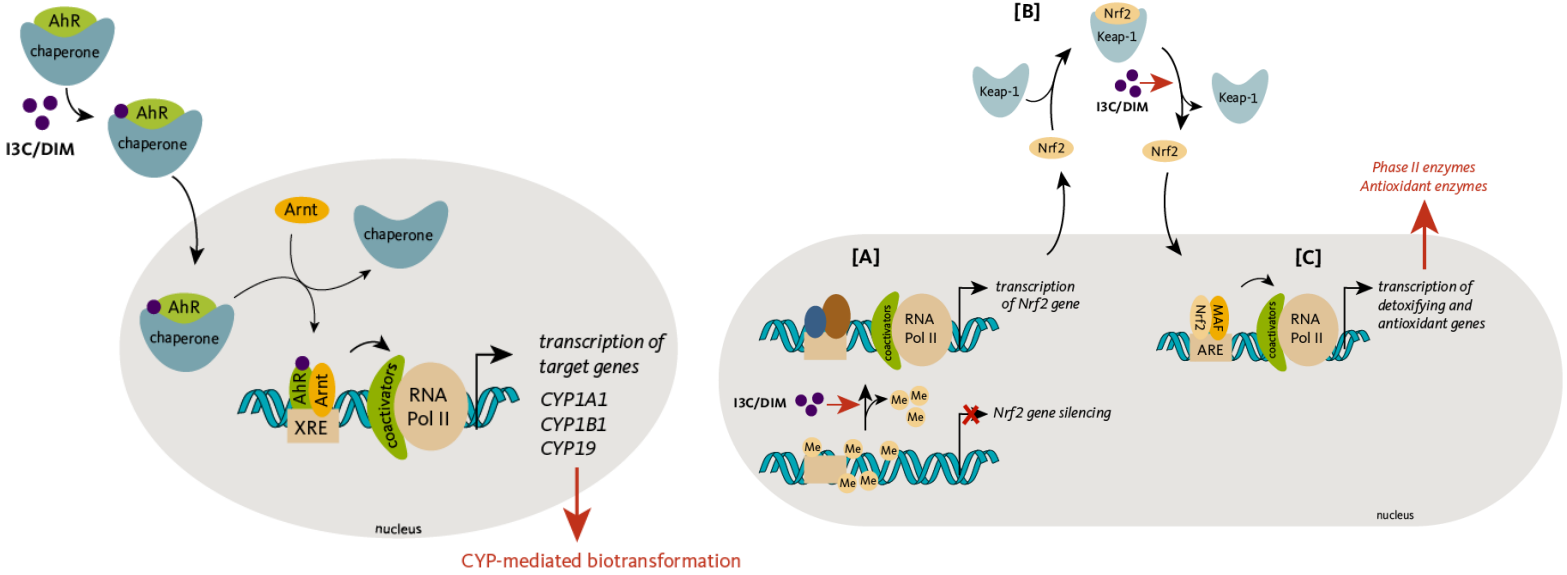
- ✓ Increased the ratio of 2-hydroxyestrones to 16α-hydroxyestrone in prostate cancer patients[7]
- ✓ Inhibitory effect on AR and AR target gene such as prostate-specific antigen (PSA)[8]
- ✓ Well-tolerated and prostate cancer patients showed detectable prostatic DIM levels[8]

1. Rajoria S et al. *Thyroid*. 2011;21(3):299-304.
2. Dalessandri KM et al. *Nutr Cancer.* 2004;50(2):161–167.
3. Ashrafian et al. *EPMA J.* 2015;6:25.
4. Zeligs M et al. *JANA.* 2005;8(1):5-15.
5. Castañon A et al. *Br J Cancer.* 2012;106(1):45–52.
6. Kotsopoulos J et al. *Br J Cancer* 2014;111(7):1269–1274.
7. Gee JR et al. *Eur J Cancer Prev*. 2016;25(4):312-320.
8. Li Y et al. *Med Princ Pract*. 2016;25(2):11–17.

Metagenics Institute

I3C and DIM regulate Phase I biotransformation enzymes via AhR signaling pathway[1]

I3C and DIM regulate Phase II biotransformation enzymes via Nrf2 signaling[2-4]



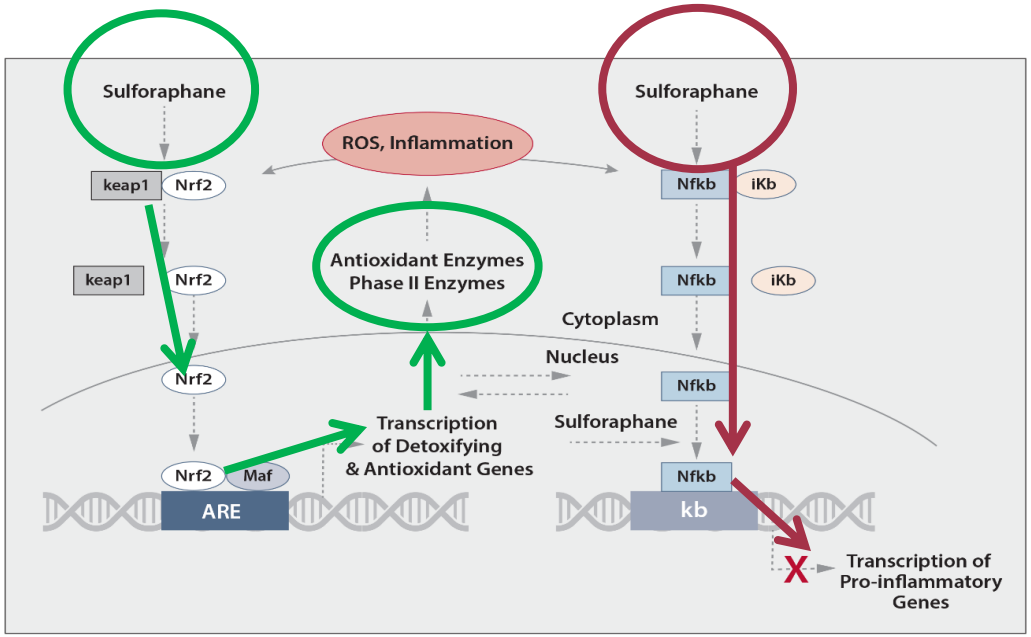
Graphics: Linus Pauling Institute. https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/indole-3-carbinol. Accessed December 12, 2018.

1. Hubbard TD et al. *Drug Metab Dispos*. 2015;43(10):1522-1535.
2. Watson GW et al. *AAPS J*. 2013;15(4):951-961.
3. Saw CL et al. *Biopharm Drug Dispos*. 2011;32(5):289-300.
4. Wu TY et al. *Mol Carcinog*. 2012;51(10):761-770.

Metagenics Institute

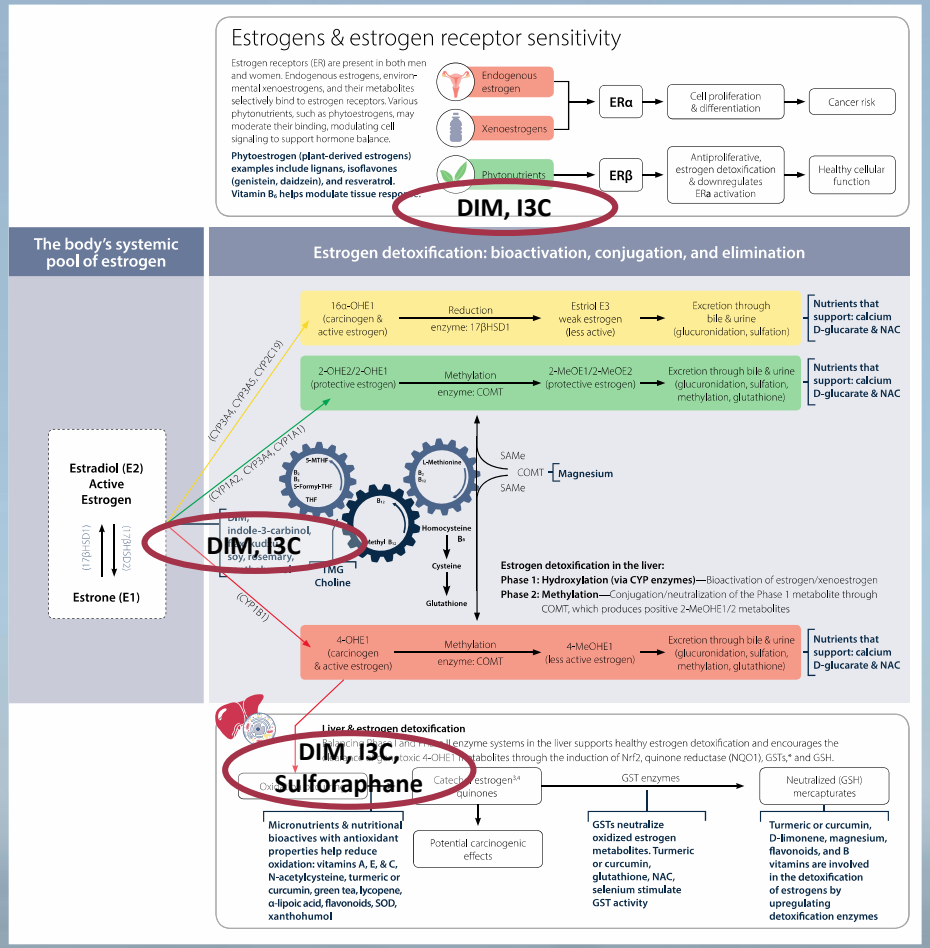
What Is the Role of Sulforaphane?

- ✓ Sulforaphane upregulates Phase II detoxification and endogenous antioxidant enzymes via Keap1-Nrf2 pathway
- ✓ It modulates NF-κB signaling pathways in inflammatory pathways



Hu R et al. *Life Sci.* 2006;79(20):1944-1955.
Bryan HK et al. *Biochem Pharmacol.* 2013;85(6):705-717.

Metagenics Institute



KEY TAKEAWAYS

DIM, I3C support both Phase I & Phase II pathways:

- Promotes 2-OH (CYP1A2, CYP3A4, CYP1A1) hydroxylation over 4-OH (CYP1B1) and/or 16α-OH hydroxylation of estrogens
- Reduces oxidized catechol estrogens by inducing Nrf2, quinone reductase (NQO1), glutathione S-transferase (GST)

Protects ERα and ERβ

Multisystem, Multitarget Other Nutritional Bioactives

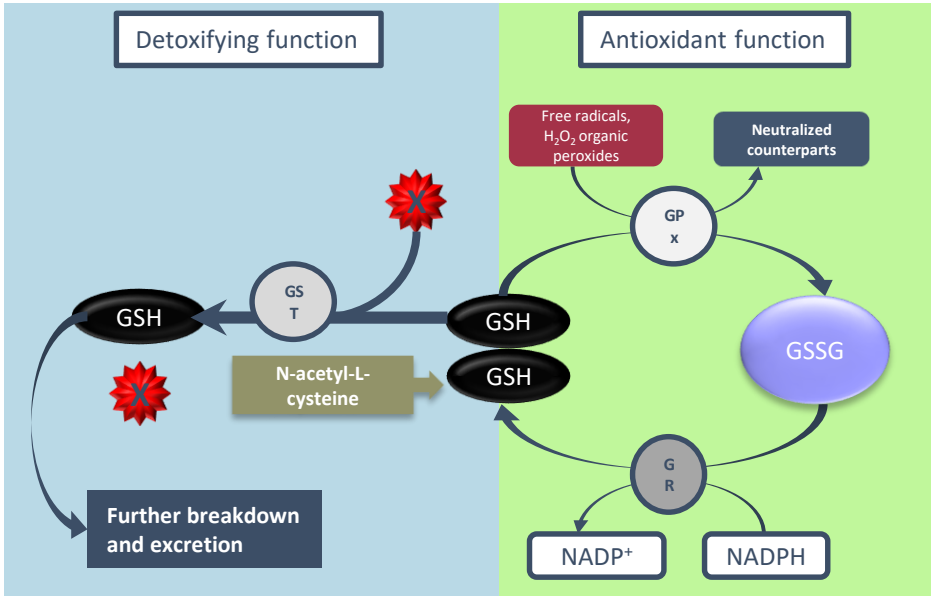
- N-acetylcysteine (NAC)
- Curcuminoids from turmeric (*Curcuma longa*)
- Green tea polyphenols (*Camellia sinensis)*
- Rosemary (*Rosmarinus officinalis)*
- Xanthohumol from hops (*Humulus lupulus*)
- Superoxide dismutase (SOD)
- Soy isoflavones
- Silymarin from milk thistle (*Silybum marianum*)

N-Acetylcysteine (NAC)

NAC is a precursor of L-cysteine, the rate-limiting factor in glutathione synthesis.

Glutathione is an important antioxidant, plays a major role in the detoxification of both endogenous and xenobiotic compounds, and is a chelating agent for heavy metals.

Improves unbalanced estrogen metabolism by reducing formation of depurinating estrogen-DNA adducts.



GST = glutathione S-transferase; **GPx** = glutathione peroxidase; **GR** = glutathione reductase

Olmstead MJ. *Altern Complemen Ther*. 2000;6(6):347-354.
Atkuri KR et al. *Curr Opin Pharmacol*. 2007;7(4):355-359.

Metagenics Institute

N-Acetylcysteine: Role in Estrogen Detoxification

- NAC as a precursor L-cysteine stimulates glutathione synthesis[1]
- Lowers lipid peroxidation[2]
- In a randomized controlled trial, administration of NAC reduced circulating homocysteine[3]
- Improves unbalanced estrogen metabolism by reducing formation of depurinating estrogen-DNA adducts[4-7]

1. Atkuri KR et al. *Curr Opin Pharmacol*. 2007;7(4):355-359.
2. Kuyumcu A et al. *Nutr J*. 2015;14:4.
3. Ventura P et al. *Pharmacol Res*. 1999;40(4):345-50.
4. Cavalieri EL et al. *Future Oncol.* 2010;6(1):75-91.
5. Zahid M et al. *Free Radic Biol Med* 2011;50(1):78–85.
6. Zahid M et al. *Free Radic Biol Med.* 2010;49(3):392–400.
7. Cavalieri EL et al. *Int J Cancer.* 2017;141(6):1078–1090.

Metagenics Institute

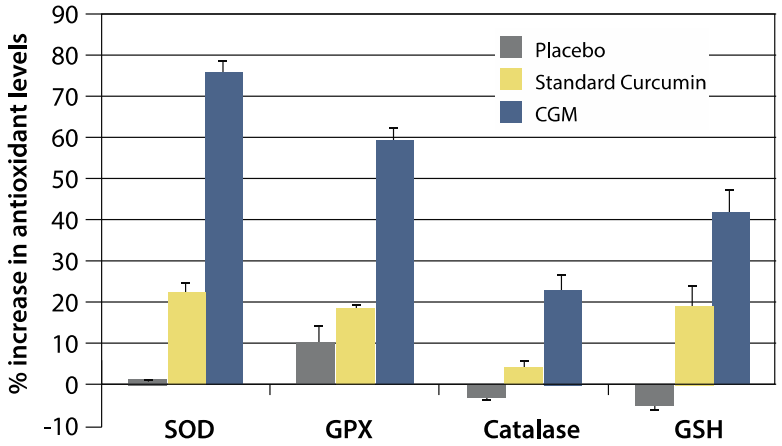
Curcuminoids: Isolated Constituents

- Primary active constituents in turmeric root (*Curcuma longa*)
- Turmeric has culinary and potential therapeutic uses
- **Properties**
  - **Analgesic**: Reduces pain including neuropathic pain in mouse models[1-2]
  - **Anti-arthritic**: e.g. Reduces joint inflammation and matrix metalloproteinase expression in mice[3]
  - **Anti-inflammatory**[4]
  - **Antioxidant**[4]
  - Other: Inflammatory bowel disease (animal models and pilot human data),[5] antidepressant (clinical data),[6,7] antidiabetic (clinical data),[8-10] cardiovascular risk markers (clinical data)[11,12]

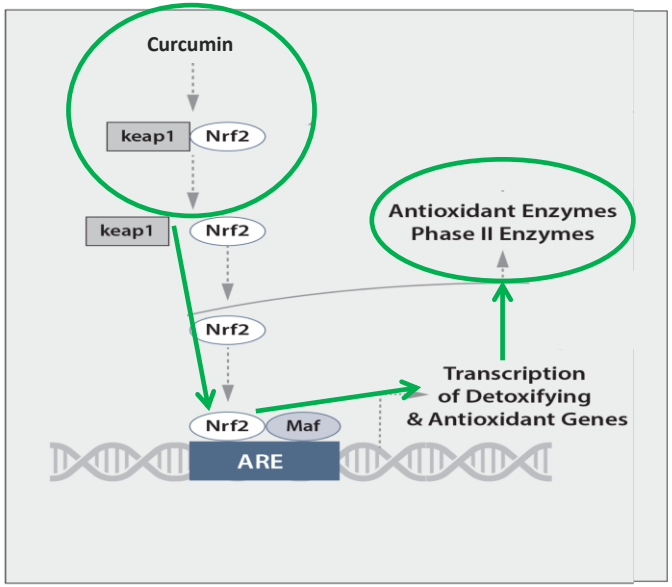
1. Banafshe HR et al. *Eur J Pharmacol.* 2014;723:202-206.
2. Zhu X et al. *PLoS One.* 2014;9(3):e91303.
3. Mun SH et al. *J Pharmacol Sci.* 2009;111(1):13-21.
4. Shezhad A et al. *J Food Sci.* 2017;82(9):2006-2015.
5. Vecchi Brumatti L et al. *Molecules.* 2014;19(12):21127-21153.
6. Sanmukhani J et al. *Phytother Res.* 2014;28(4):579-585.
7. Lopresti AL et al. *J Affect Disord.* 2014;167:368-375.
8. Maradana MR et al. *Mol Nutr Food Res.* 2013 ;57(9):1550-1556.
9. Na LX et al. *Mol Nutr Food Res.* 2013;57(9):1569-1577.
10. Chuengsamarn S et al. *Diabetes Care.* 2012;35(11):2121-2127.
11. Khurana S et al. *Nutrients.* 2013;5(10):3779-3827.
12. Chuengsamarn S et al. *J Nutr Biochem.* 2014;25(2):144-150.

Metagenics Institute

Curcumin Beneficially Influences the Activity of Phase II Detoxification Enzymes



Adapted from: Pandaran Sudheeran S et al. *J Clin Psychopharmacol.* 2016;36(3):236-243.



Iqbal M et al. *Pharma & Toxicol.* 2003;92(1):33–38.
Jagetia GC et al. *Antioxidants (Basel).* 2015;4(1):25-41.
Wu KC et al. *Planta Med*. 2014;80(1):97–104.
Kelsey NA et al. *Molecules.* 2010;15(11):7792–7814.
Levine CB et al. *BMC Vet Res.* 2017;13:388.
Singh SV et al. *Carcinogenesis.* 1998;19(8):1357–1360.
Valentine SP et al. *Life Sci.* 2006;78(20);2391-2398.
Wu J et al. *PLoS One.* 2013;8(3):e59843.
Goud VK et al. *Plant Foods Hum Nutr.* 1993;44(1):87-92.
Susan M et al. *Arzneimittelforschung.* 1992;42(7):962-964.

Metagenics Institute

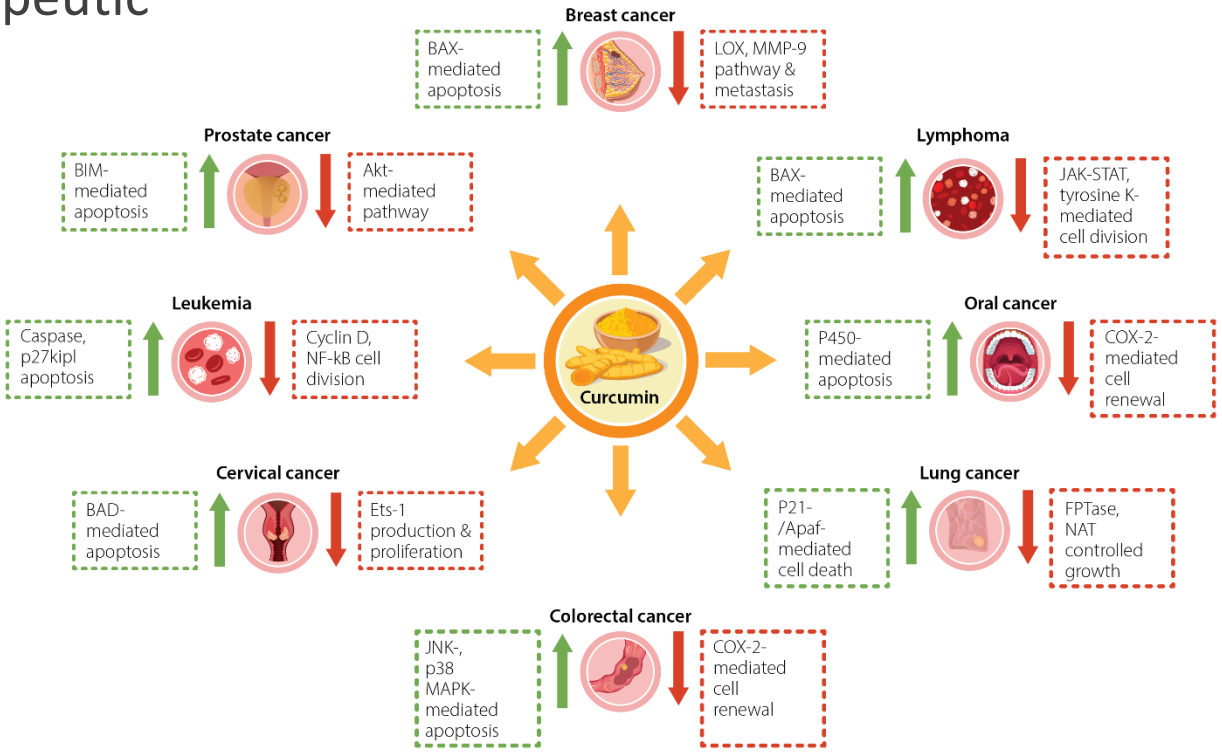
Curcumin and Breast Cancer: Preclinical Evidence

- Curcumin reduces xenoestrogen-induced growth of breast cancer cell proliferation and apoptosis *in vitro* and inhibits tumor growth and angiogenesis *in vivo*.[1,2]
- Curcumin and epigallocatechin gallate (EGCG) singly and in combination synergistically suppresses ERα-breast cancer cell growth *in vitro* and *in vivo*.[3,4]

1. Palange AL et al. *Front Oncol*. 2012;2:161.
2. Bimonte S et al. *Biomed Res Int.* 2015;2015:878134.
3. Somers-Edgar TJ et al. *Int J Cancer.* 2008;122(9):1966–1971.
4. Khafif A et al. *Carcinogenesis.* 1998;19(3):419–424.

New Insights into Therapeutic Activity and Anticancer Properties of Curcumin

Curcumin has anticarcinogenic activities in the areas of cell transformation, proliferation, apoptosis, survival, invasion, metastasis, and adhesion, as well as angiogenesis.



Adapted from: Panda AK et al. *J Exp Pharmacol.* 2017;9:31–45.

Metagenics Institute

Tea Polyphenols in Promotion of Human Health

- The three major forms of tea are green, black, and oolong tea based on the degree of fermentation.[1]
- Polyphenols are the major active compounds present in teas.
- The catechins are the major polyphenolic compounds in green tea, and epigallocatechin-3-gallate (EGCG) is the predominant and most studied catechin in green tea.[1]
- Research indicates a potential role of tea polyphenols in the prevention of cancer, diabetes, cardiovascular and neurological diseases.[1-3]

1. Khan N et al. *Nutrients*. 2019;11(1):39.
2. Negri A. et al. *Nutrients.* 2018;10(12):E1936.
3. Al Hroob AM. Et al. *Biomed Pharmacother.* 2019;109:2155-2172.

Metagenics Institute

Properties of Green Tea Polyphenols

- Antioxidative[1]
  - Antioxidant potential of EGCG believed to be far greater than vitamins E and C
  - Anti-inflammatory effects
- Detoxifying[2]
  - Supports glucuronidation of estrogen
  - Inhibits activation of carcinogens by CYP450 enzymes
- Anticarcinogenic[3-4]
  - Results in apoptosis of several cancer cells, but not of normal cells
  - Inhibition of activities related to tumor promotion, proliferation, and mitotic signal transduction

1. Bernatoniene J et al. *Molecules*. 2018;23(4):965.
2. Mohamed ME et al. *Drug Metab Dispos.* 2011;39(9):1522-1528.
3. Feitelson MA et al. *Semin Cancer Biol*. 2015;35 Suppl:S25–S54.
4. Gianfredi V et al. *J Nutrigenet Nutrigenomics.* 2017;10(3-4):126–135.

Metagenics Institute

Regularly Drinking Green Tea May Favorably Influence Estrogen Metabolism

Study objective: Identify how green tea polyphenols, which may reduce breast cancer risk, can potentially influence enzymes involved in estrogen metabolism

Design:
Green tea intake (< 1 time/week, 1-6 times/week, or 7+ times/week) was assessed in a group of healthy Japanese American women, 119 of which were in the luteal phase and 72 of which were postmenopausal

Results:
Green tea intake was correlated with lower urinary estrogens and estrogen metabolites (including 16-pathway metabolites) in premenopausal women. Urinary estradiol and estrone were ~40% and ~20% lower in postmenopausal women who drank green tea daily, in comparison to those who drank green tea < 1 time/week

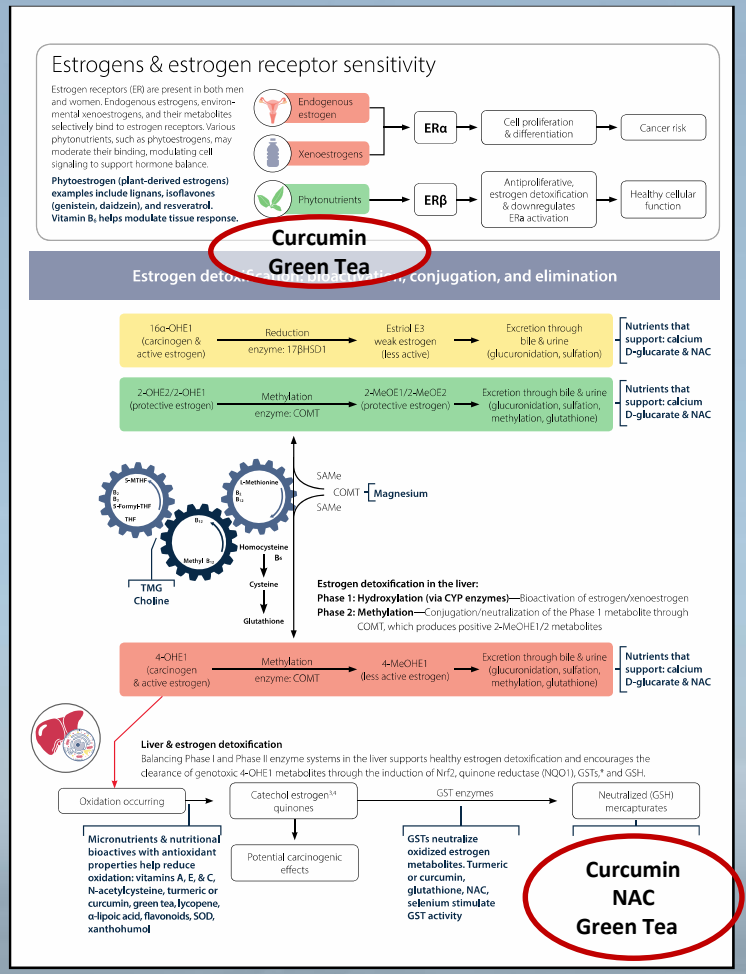
Conclusion: Green tea is a rich source of phytochemicals that can interact with and regulate xenobiotic metabolizing enzymes, and green tea may modify metabolism or conjugation of estrogens.

Fuhrman BJ et al. *Nutr J.* 2013;12:25.

Rosemary (*Rosmarinus officinalis*) and Estrogen Detoxification

- Rosemary is a common culinary herb, which is rich in polyphenols including rosmarinic and carnosic acids.[1]
- Rosemary promotes the 2-hydroxylation of estrogen and may inhibit 16α-hydroxylation.[2]
- Rosemary may also enhance estrogen detoxification.[2] Turmeric root and rosemary leaf extracts can work synergistically to reduce neoplastic cell growth.[3-4]
- Carnosic acid and carnosol, two major constituents of rosemary, show antioxidant, anti-inflammatory, and anticancer properties.[5-6]

1. Moore J et al. *Nutrients*. 2016;8(11);E731.
2. Zhu BT et al. *Carcinogenesis.* 1998;19(10):1821-1827.
3. Einbond LS et al. *Fitoterapia*. 2012;83(7):1160-1168.
4. Levine CB et al. *BMC Vet Res*. 2017;13:388.
5. Loussouarn M et al. *Plant Physiol*. 2017;175(3):1381-1394.
6. Moore J et al. *Nutrients.* 2016;8(11):E731.



KEY TAKEAWAYS

Curcumin & green tea

Positive synergistic influence on ERα and Erβ

Curcumin, green tea, rosemary, NAC

Reduce oxidation of catechol estrogens (2-OH and 4-OH) to catechol estrogen quinones and formation of depurinating DNA adducts by inducing Nrf2, quinone reductase (NQO1), heme-oxygenase (HO), and glutathione S-transferase (GST)

Xanthohumol Modulates the Chemical Estrogen Carcinogenesis Pathway

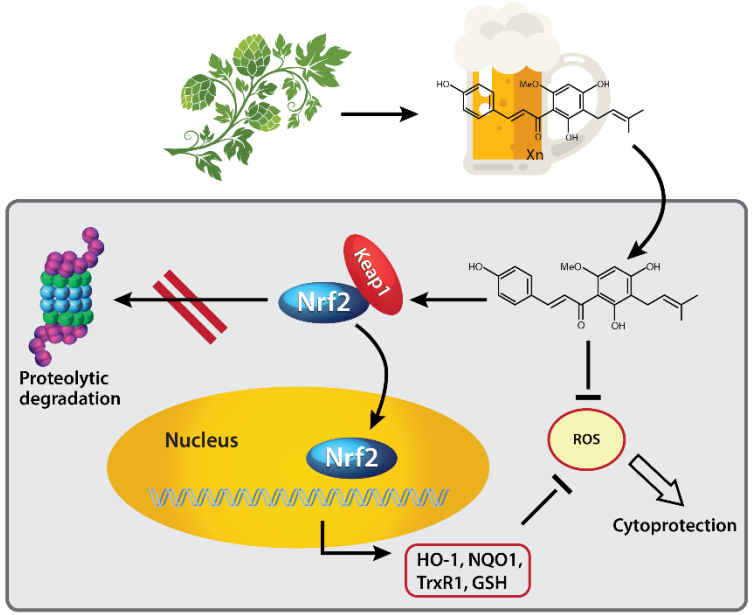
- Xanthohumol—one of the constituents in hops flowers (*Humulus lupulus*)
- Xanthohumol increases 2-hydroxylation and decreases 4-hydroxylation metabolism, which may play a protective role against breast cancer processes
- In both hormone-sensitive and hormone-refractory human breast and prostate cancer cells, xanthohumol:
  - Inhibits the growth and induced apoptosis
  - Inhibits prosurvival Akt, NF-κB, and mTOR signaling proteins and NF-κB-regulated antiapoptotic Bcl-2 and surviving
  - Inhibits the proliferation of ERα-positive breast cancer cells

Wang S et al. *Chem Res Toxicol*. 2016;29(7):1142−1150.
Deeb D et al. *Anticancer Res.* 2010;30(9):3333–3339.
Yoshimaru T et al. *Sci Rep*. 2014;4:7355

Dietary Xanthohumol Influences the Regulation of Detoxifying Enzymes in Liver and Mammary Gland Tissues

Xanthohumol

- Induces Phase II detoxification and endogenous antioxidant enzymes
- Activates Nrf2 enzymes to confer protection against oxidative damage in breast and also prostate cells



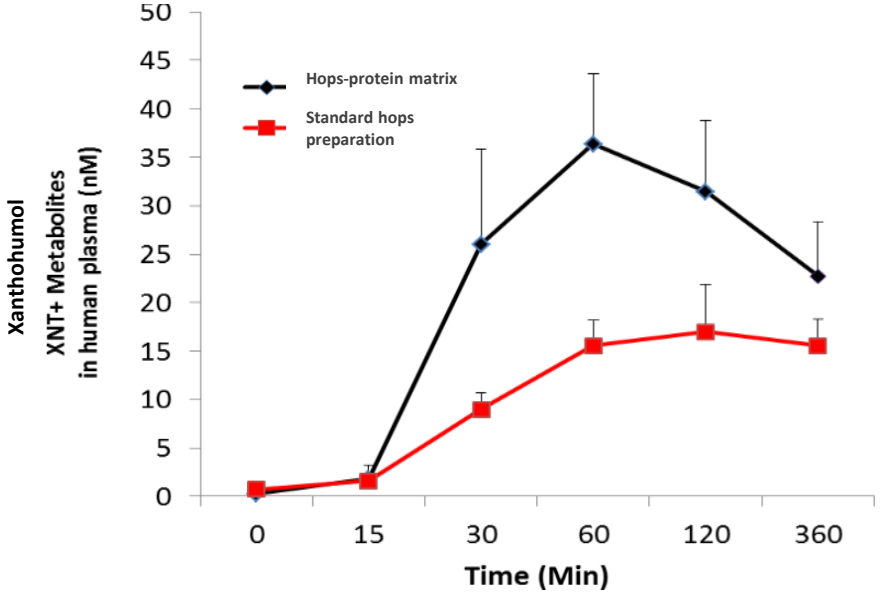
Adapted from: Yao J et al. *J Agric Food Chem*. 2015;63(5):1521−1531.

Ferk F et al. *Mol Nutr Food Res*. 2016;60(4):773–786.
Pichler C et al. *Cancer Prev Res (Phila)*. 2016;10(2):153–160.

Metagenics Institute

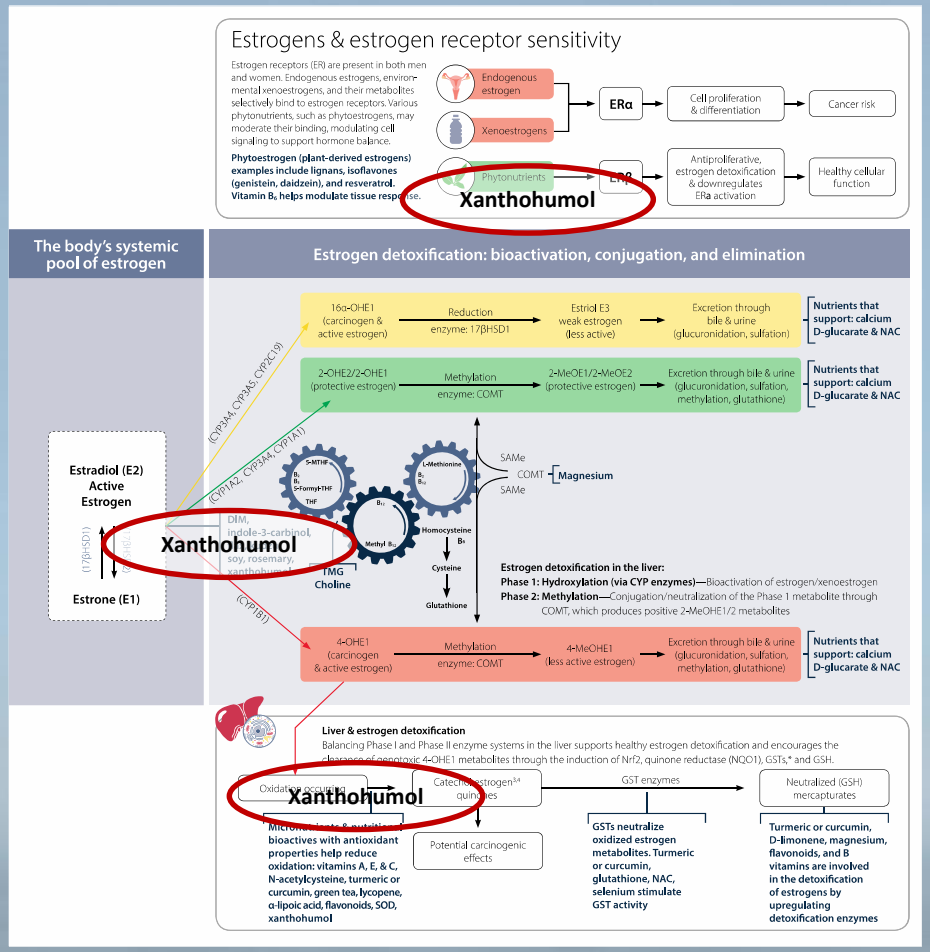
Xanthohumol Availability Is Enhanced by Delivery Through a Hops-Protein Matrix

- Xanthohumol is generally not well absorbed
- Xanthohumol given as part of a hops-protein matrix shows enhanced bioavailability compared to standard hops-xanthohumol preparations



Adapted from: O'Connor A et al. *Mol Nutr Food Res*. 2018;62(6):e1700692.

Metagenics Institute



KEY TAKEWAYS

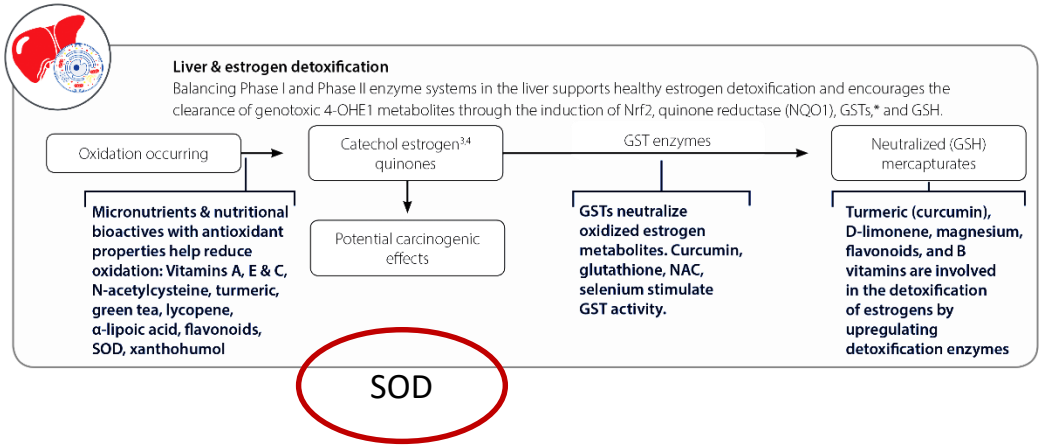
Xanthohumol

hops-protein matrix enhanced bioavailability

- Increases 2-OH and decreases 4-OH in Phase I metabolism, which may play a protective role against breast cancer processes
- Induces Phase II detoxification and endogenous antioxidant enzymes
- Activates Nrf2 enzymes to confer protection against oxidative damage in breast and also prostate cells

Superoxide Dismutase (SOD) for oxidative stress and Phase II Detoxification

- ✓ Antioxidant enzyme superoxide dismutase (SOD) is an enzyme found in all living cells
- ✓ SOD derived from French melon been studied for its interaction with the body's endogenous primary antioxidant enzymes: SOD, CAT, and GPx.
- ✓ SOD derived from French melon has also been shown to upregulate phase II detoxification and antioxidant enzymes.



Milesi MA et al. *Nutr J*. 2009;15:8:40.
Carillon J et al. *Nutrients.* 2014;19(6):2348-2359

Metagenics Institute

Soy and Soy Isoflavones

- Isoflavones are the major flavonoids found in legumes, particularly soybeans.[1,2]
- Isoflavones are classified as phytoestrogens—plant-derived compounds with estrogenic activity. [2]
- Soy isoflavones are known to have weak estrogenic or hormone-like activity due to their structural similarity with 17-β-estradiol. [2]
- The average isoflavone intake among adults ranges from about 30–50 mg/day in Japan but is < 3 mg/day in the US, Canada, and

1. Franke AA et al. *Arch Biochem Biophys.* 2014;559:24-28.
2. Lampe JW *J Nutr.* 2003;133 Suppl 3:956S-964S.
3. Bai W et al. *Int J Food Sci.* 2014;6:9-14

Soy and Soy Isoflavones: Benefits in Estrogen Metabolism, Breast, & Prostate Health

Improved ratio of 2-OH to 16α-OH estrogens and increases SHBG	• Randomized studies in premenopausal women showed significant increase in urinary 2:16α-OH ratios and lower serum estradiol levels with the consumption of soy products.[1-3] • Women with low levels of SHBG, consumption of a soy milk powder providing about 69 mg of soy isoflavones daily substantially increased their SHBG concentrations.[4]
Estrogen receptor selectivity	• Soy isoflavones can preferentially bind to and transactivate ERβ—rather than ERα—mimicking the effects of estrogen in some tissues and antagonizing (blocking) the effects of estrogen in others.[5]
Higher intakes of soy and lower risk of breast cancer	• Higher intakes of soy products and isoflavones are associated with low rates of hormone-dependent cancers.[4] • In a large ethnically diverse cohort of women with breast cancer living in North America, a higher dietary intake of isoflavone was associated with reduced all-cause mortality. • Higher intakes of soy foods early in life may decrease the risk of breast cancer in adulthood.[6-10]
Reduced risk of prostate cancer	• Supplementation with soy products, soy dietary proteins, or soy isoflavones could reduce or slow down the rising of serum PSA concentration in men with localized prostate cancer prior to therapy, reduced the risk of prostate cancer as well as in those with PSA biochemical recurrence following radiotherapy and/or prostatectomy.[11-13]

1. Morimoto Y et al. *Nutr Cancer.* 2012;64(2):307–314.
2. Lu LJ et al. *Cancer Epidemiol Biomarkers Prev.* 1996;5(1):63-70.
3. Xu X et al. *Cancer Epidemiol Biomarkers Prev.* 1998;7(12):1101-1108.
4. Pino AM et al. *J Clin Endocrinol Metab.* 2000;85(8):2797-2800.
5. Wang LQ. J Chromatrogr B Analyt Technol Biomed Life Sci. 2002;777(1-2):289-309.
6. He F et al. *Food Sci and Human Well.* 2013;2:146–161.
7. Li Y et al. *Nutrients.* 2017;9(7):728.
8. Zhang FF et al. *Cancer.* 2017;123(11):2070–2079.
9. Vitale DC et al. *Eur J Drug Metab Pharmacokinet.* 2013;38(1):15–25.
10. Cojocneanu PR et al. *Onco Targets Ther.* 2015;8:2053–2066.
11. Zhang M et al. *Andrology.* 2016;4(4):745-756.
12. Lazarevic B et al. *Nutr Cancer.* 2011;63(6):889-898.
13. Kwan W et al. *Nutr Cancer.* 2010;62(2):198-207.

Metagenics Institute

Milk Thistle

- Silymarin is a natural compound that is present in species derived from *Silybum marianum*, which is commonly known as milk thistle.[1,2]
- Milk thistle contains at least seven flavolignans and the flavonoid taxifolin.[1]
- The most important flavolignan, silybin represents between 50% and 70% of the extract from silymarin.[2]
- Silymarin has both hepatoprotective and regenerative actions.[2]

1. Vargas-Mendoza N et al. *World J Hepatol.* 2014;6(3):144-149.
2. Loguercio C et al. *World J Gastroenterol.* 2011;17(18):2288-2301

Silymarin (Milk Thistle)—Hepatoprotection

Silymarin can mitigate oxidative stress, fibrosis, cirrhosis, and lipid peroxidation.[1]

Patients with non-alcoholic steatohepatitis (NASH) treated with silymarin showed reduced liver enzyme (ALT and AST) levels.[2-3]

Silymarin and milk thistle show hepatoprotective effects.[4-5]

Antioxidant	Proposed Degree of Hepatoprotective Effect
Silymarin	**The highest**
Green tea	Lower than silymarin
Quercetin	Lower than green tea
Curcumin	Lower than quercetin
Resveratrol	Lower than curcumin
Naringenin	Lower than resveratrol
Coffee	The lowest

Adapted from: Casas-Grajales S et al. *World J Gastrointest Pharmacol Ther.* 2015;6(3):59-72.

1. Casas-Grajales S et al. *World J Gastrointest Pharmacol Ther.* 2015;6(3):59-72.
2. Masoodhi M et al. *Govaresh.* 2013;18(3):181-185.
3. Solhi H et al. *Caspian J Intern Med.* 2014;5(1):9-12.
4. Vargas-Mendoza N et al. *World J Hepatol*. 2014;6(3):144-149.
5. Abenavoli L et al. *Phytother Res*. 2010;24(10):1423-32.

Gut-Estrogen Connection Targeted Bioactive Support

- Calcium D-glucarate inhibits β-glucuronidase—the enzyme produced by colonic microflora that ultimately results in potentially increasing the risk of carcinogenesis[1]
- Probiotics and prebiotics promote a healthy microbiome and estrobolome—the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens that might affect women's risk of developing postmenopausal estrogen receptor-positive breast cancer[2]

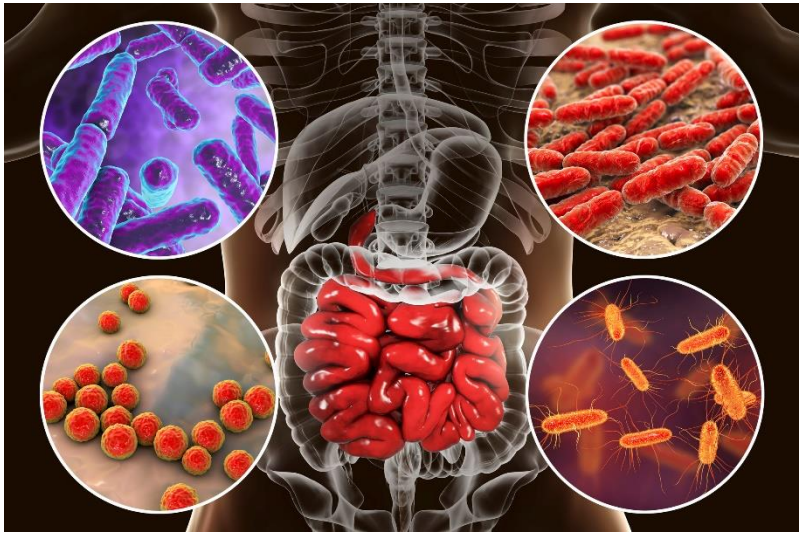
1. Walaszek K et al. *Cancer Detect Prev.* 1997;21(2):178-190.
2. Baker JM et al. *Maturitas*. 2017;103:45-53.

Metagenics Institute

The Microbiome

"Humans and microbes have coevolved a complex intricate relationship to benefit the host while allowing the intestinal microbiota to thrive in a mutually advantageous equilibrium."

"Microbiome perturbation can, however, be associated with risk of developing inflammatory, autoimmune, and malignant disease. Microbial community dysbiosis, a pathologic disequilibrium, could potentially favor oncogenesis and tumor progression and affect responses to cancer therapy and toxicity profiles of chemotherapeutics."



Kwa M et al. *J Natl Cancer Inst*. 2016;108(8):djw029.

Different Bacterial Profiles in Breast Tissue Exist Between Healthy Women and Those with Breast Cancer

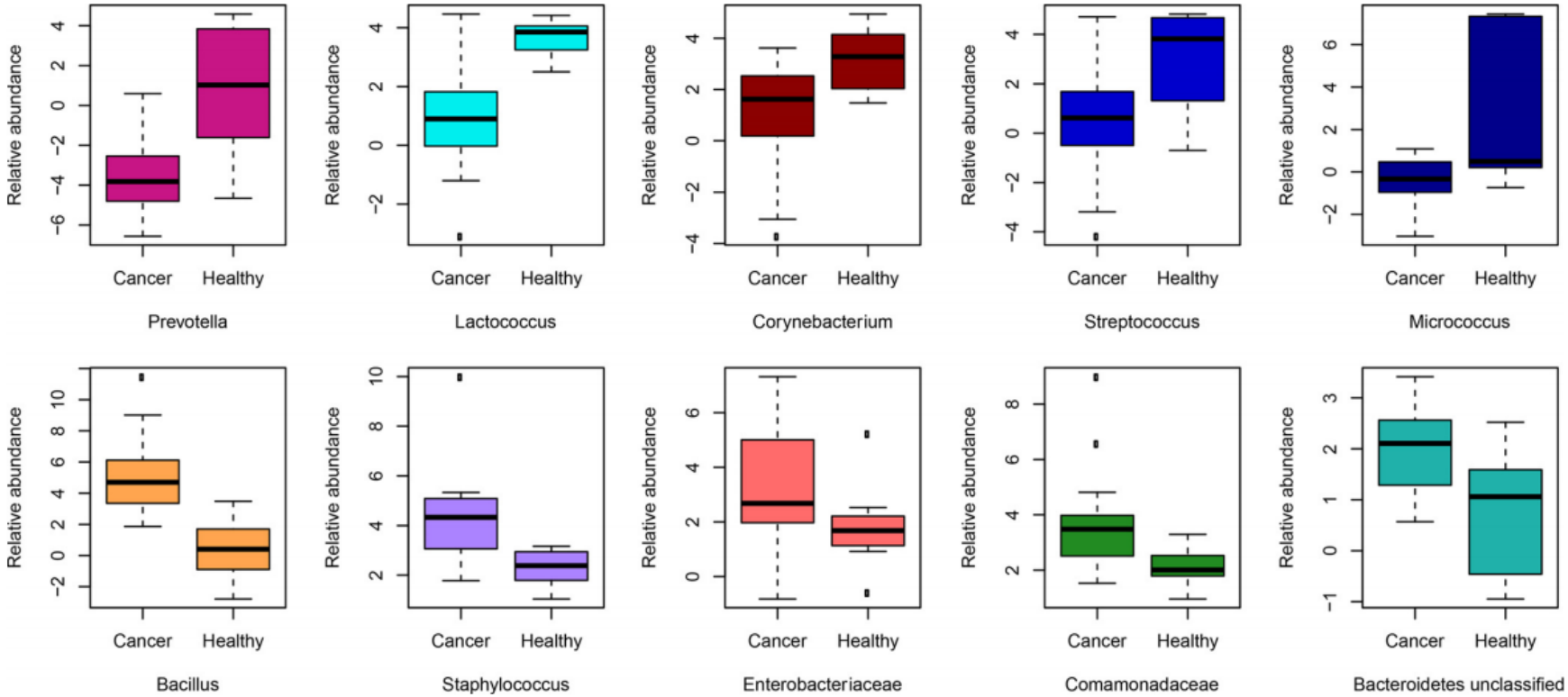


Figure: Urbaniak C et al. *Appl Environ Microbiol.* 2016;2(16):5039-5048.
http://creativecommons.org/licenses/by/4.0/. Accessed February 28, 2019.

Metagenics Institute

D-Glucarate

- D-glucarate, also known as glucaric acid, naturally occurs in grapefruit, oranges, and apples[1]
- Once consumed, D-glucarate is metabolized into derivatives including D-glucaro-1,4-lactone[2]
- D-glucaro-1,4-lactone suppresses β-glucuronidase activity[3]

1. Dwivedi C et al. *Biochem Med Metab Biol*. 1990;43(2):83-92.
2. Walaszek K et al. *Cancer Detect Prev*. 1997;21(2):178-190.
3. Macfadyen A et al. *Hepatology*. 1989,9(4):552-556.

Metagenics Institute

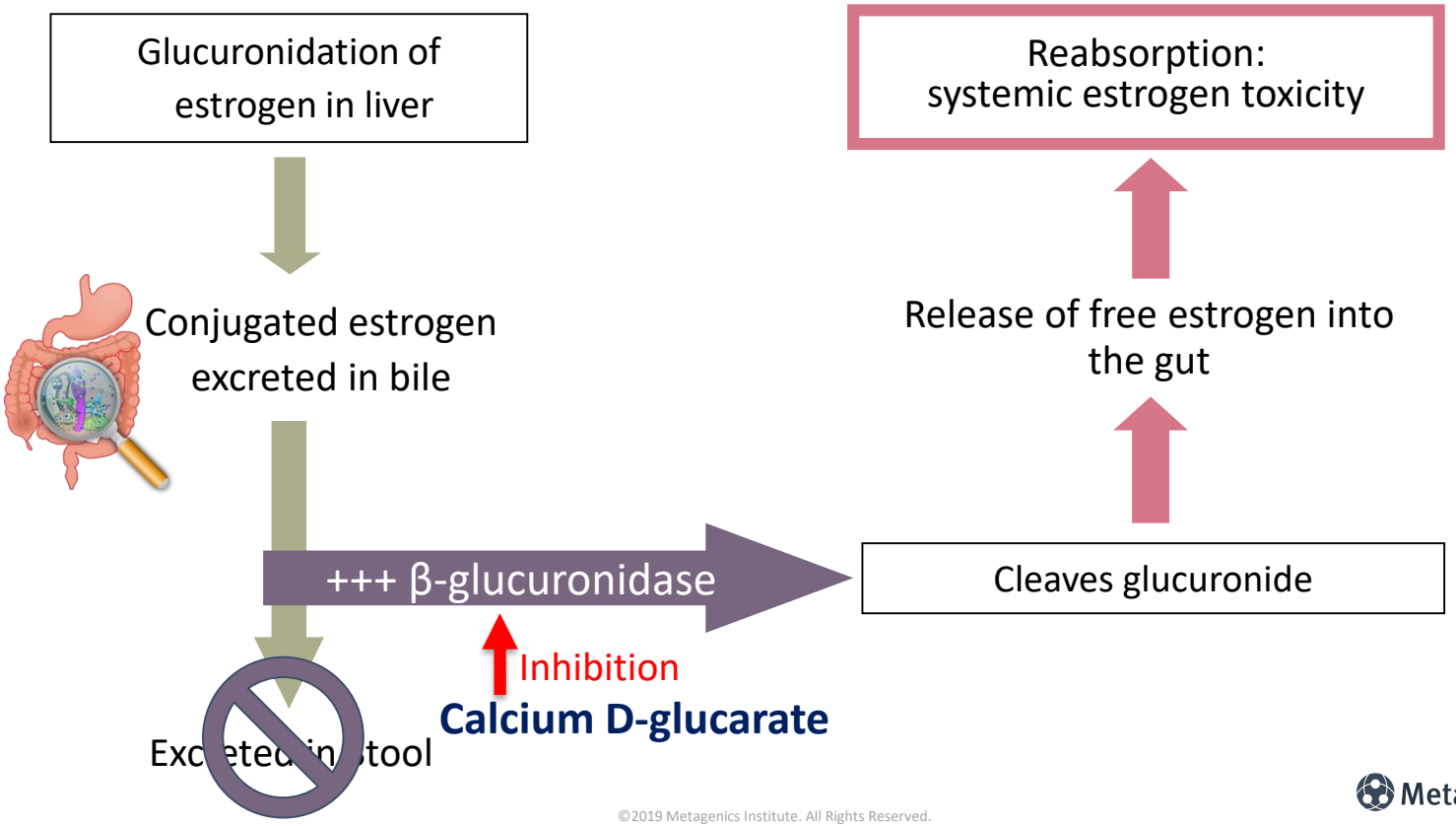
Detoxifying and Anticarcinogenic Properties of Calcium D-Glucarate

- Inhibits β-glucuronidase, an enzyme produced by colonic microflora. Inhibition of β-glucuronidase ultimately results in potentially decreasing the risk of carcinogenesis.[1,2]
- Involved in Phase II liver detoxification – glucuronidation.
- A cross-sectional study among 279 healthy men and women demonstrated that several dietary factors like higher intakes of calcium, iron, and magnesium and demographic factors like being male, older age, and overweight were associated with β-glucuronidase activity.[3]
- A commonly recommended oral dose of calcium D-glucarate in clinical settings is 1,500-3,000 mg daily.[2]

1. Dwivedi C et al. *Biochem Med Metab Biol*. 1990;43(2):83-92.
2. Walaszek K et al. *Cancer Detect Prev*. 1997;21:178-190.
3. Maruthi SS et al. *Nutr Cancer*. 2010;62(2):208–219.

Role of Calcium D-Glucarate

Phase II glucuronidation and estrogen excretion



Metagenics Institute

The Estrobolome: Gut Microbes Affect Estrogen Metabolism & Cancer Risk in Animal Models

- Diet supplementation with the probiotic strain ***Lactobacillus acidophilus*** **NCFM™** significantly suppressed **colon cancer risk in rats** treated with azoxymethane. A significant dose-dependent **reduction of cecal β-glucuronidase** activities was observed.[1]
- **Minipigs** fed with high-cholesterol diet for a baseline period, followed by the diet containing a mixture of **three *Lactobacillus* strains** significantly reduced the fecal enzyme activity for β-glucuronidase activity. [2]

1. Rao CV et al. *Int J Oncol.* 1999;14(5):939-944.
2. Haberer P et al. *Int J Food Microbiol.* 2003;87(3):287–291.

KEY TAKEAWAYS

Calcium D-Glucarate, Probiotics, Prebiotics, Lignans, Fiber

- Discourages dysbiosis by **supporting a healthy microbiome and estrobolome**
- **Inhibits β-glucuronidase**, an enzyme produced by colonic microbiota associated with breast cancer incidence
- Supports the **excretion of excess estrogens through glucuronidation**—Phase II liver detoxification.

Estrobolome—gut & estrogen connection

The gut plays a vital role in the body's ability to clear unwanted estrogen metabolites. Gut dysbiosis can contribute to β-glucuronidase activity, which allows estrogen to reenter circulation and increase the estrogen pool. Effects can be unbalanced hormones. Proper gut health, nutrition, and the types and amounts of macronutrients support estrogen biotransformation and clearance.

5R gut protocol, probiotics, prebiotics, fiber (apple pectin)	β-glucuronidase inhibitors: probiotic strain *L. acidophilus* NCFM®, calcium D-glucarate; lignans	Flax lignans support excretion

Clinical Considerations for Estrogen Metabolism Support

- Decrease total exposure to endocrine-disrupting chemical (EDCs)[1]
- Provide low allergenicity proteins and quality healthy fats to support the high energy requirements of detoxification, and to support glucose balance[2,3]
- Promote insulin sensitivity
- Manage mental and emotional stressors
- Support the gut microbiome and fecal elimination

1. Diamanti-Kandarakis E et al. *Endocr Rev*. 2009;30(4):293–342.
2. Hammond GL. *J Endocrinol*. 2016; 230(1):R13-25.
3. Zand H. *Diabetes Metab Syndr*. 2017;11 Suppl 1:S307-S309.

Metagenics Institute

Choose Foods and Cofactors That…

- Inhibit the activity of β-glucuronidase, which deconjugates estrogens in the large intestine, allowing them to be reabsorbed and remetabolized[1]
- Inhibit the activity of aromatase, which converts prohormones into estrogens in extra-gonadal tissues[2]
- Increase circulating concentrations of SHBG, thus reducing levels of unbound, active estrogens[3]
- Modify estrogen receptor activity in favor of ERβ with phytoestrogens[4]
- Promote C-2 hydroxylation over C-4 and/or C-16α hydroxylation of estrogens[5]
- Reduce the oxidation of catechol estrogens (2-OH and 4-OH)[6]
- Promote the methylation of catechol estrogens (2-OH and 4-OH)[7]
- Promote the detoxification of estrogens by upregulating Phase I and Phase II enzymes[8,9]

1. Plotnikoff GA. *Glob Adv Health Med*. 2014;3(3):33-43.
2. Miller WL et al. *Endocr Rev.* 2011;32(1):81–151.
3. Hammond GL. *J Endocrinol*. 2016;230(1):R13-25.
4. Shanle EK et al. *Chem Res Toxicol*. 2011;24(1):6-19.
5. Muti P et al. *Epidemiology*. 2000;11(6):635-640.
6. Cavalieri E et al. *Mol Aspects Med*. 2014;36:1–55.
7. Hodges RE et al. *J Nutr Metab*. 2015;2015:760689.
8. Tsuchiya Y et al. *Cancer Lett.* 2005;227(2):115–124.
9. Bock KW. *Biochem Pharmacol*. 2015;96(2):77-82.

Metagenics Institute

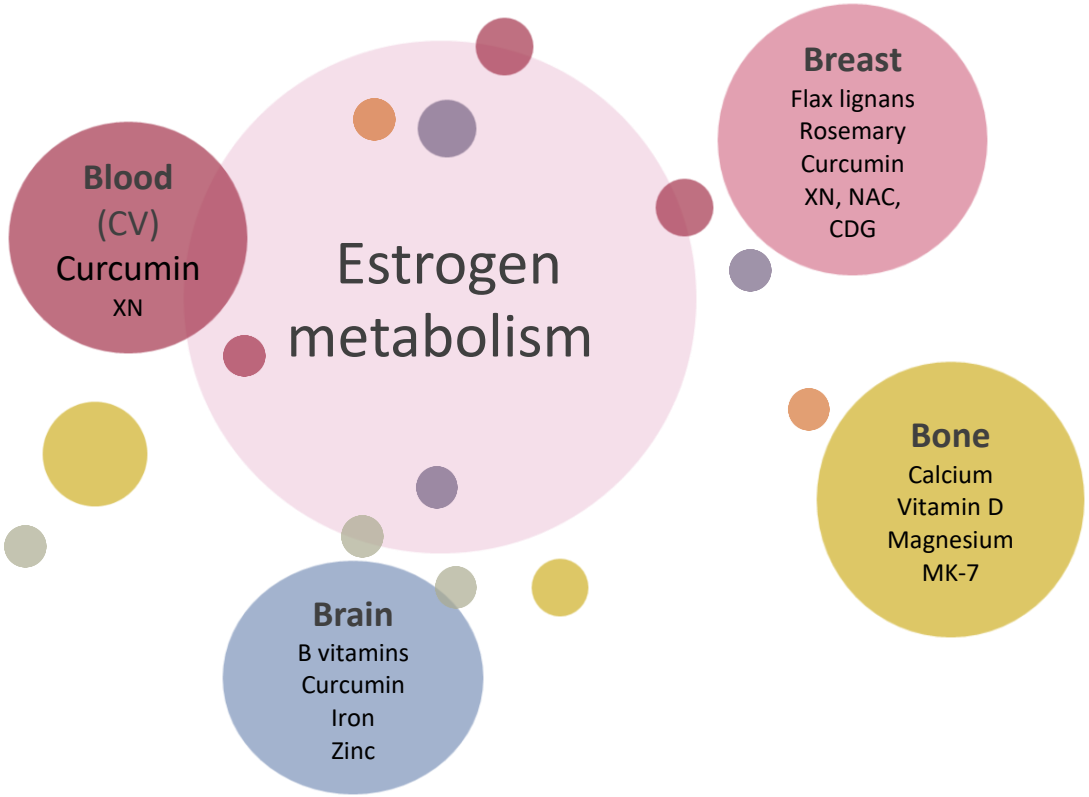
HPG Imbalance Is Associated with Estrogen Burden and Affects How Women Feel, Think, and Behave

Common physical complaints	Emotional & mood-related symptoms
• Thirst and appetite changes (food cravings) • Breast tenderness • Bloating and weight gain • Headache • Swelling of the hands or feet aches and pains • Fatigue • Skin problems • Gastrointestinal symptoms • Painful periods and abdominal pain • Lower tolerance for noise or light	• Depression • Anxiety • Angry outbursts • Irritability • Crying spells • Anxiety • Confusion • Social withdrawal • Poor concentration • Insomnia • Increased nap taking • Changes in sexual desire • Trouble sleeping

https://www.womenshealth.gov/menstrual-cycle/premenstrual-syndrome Accessed on January 23, 2019.
Del Río JP et al. *Front Public Health.* 2018;6:141.
Raghunath RS et al. *Clin Exp Dermatol*. 2015;40(2):111-115.
Nie X et al. *Dig Dis Sci*. 2018;63(3):583-596.
Martín-Millán M et al. *Joint Bone Spine*. 2013;80(4):368-373.

Metagenics Institute

Estrogen Metabolism Influences Nonhormonal Tissues



Metagenics Institute

Thank you.

Metagenics Institute