Estrogen Metabolism:
Are We Assessing It Properly?

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Technical Issues & Clinical Questions

Please type any technical issue or clinical question into either the “Chat” or “Questions” boxes, making sure to send them to “Organizer” at any time during the webinar.

We will be compiling your clinical questions and answering as many as we can the final 15 minutes of the webinar.

DISCLAIMER: *Please note that any and all emails provided may be used for follow up correspondence and/or for further communication.*

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Estrogen Metabolism:
Are We Assessing It Properly?

Filomena Trindade, MD, MPH

Objectives

- Understand the importance of estrogen metabolism with respect to cancer risk
- Be able to devise a treatment plan for a pt with an unfavorable estrogen metabolism profile
- Gain a basic understanding of the importance of methylation in estrogen metabolism, health promotion and cancer prevention
- Learn to apply these principles in the clinical setting in order to assess potential cancer risk in men and women
- Gain knowledge on the relevance of estrogen metabolism and detoxification in hormone related cancers in both men and women

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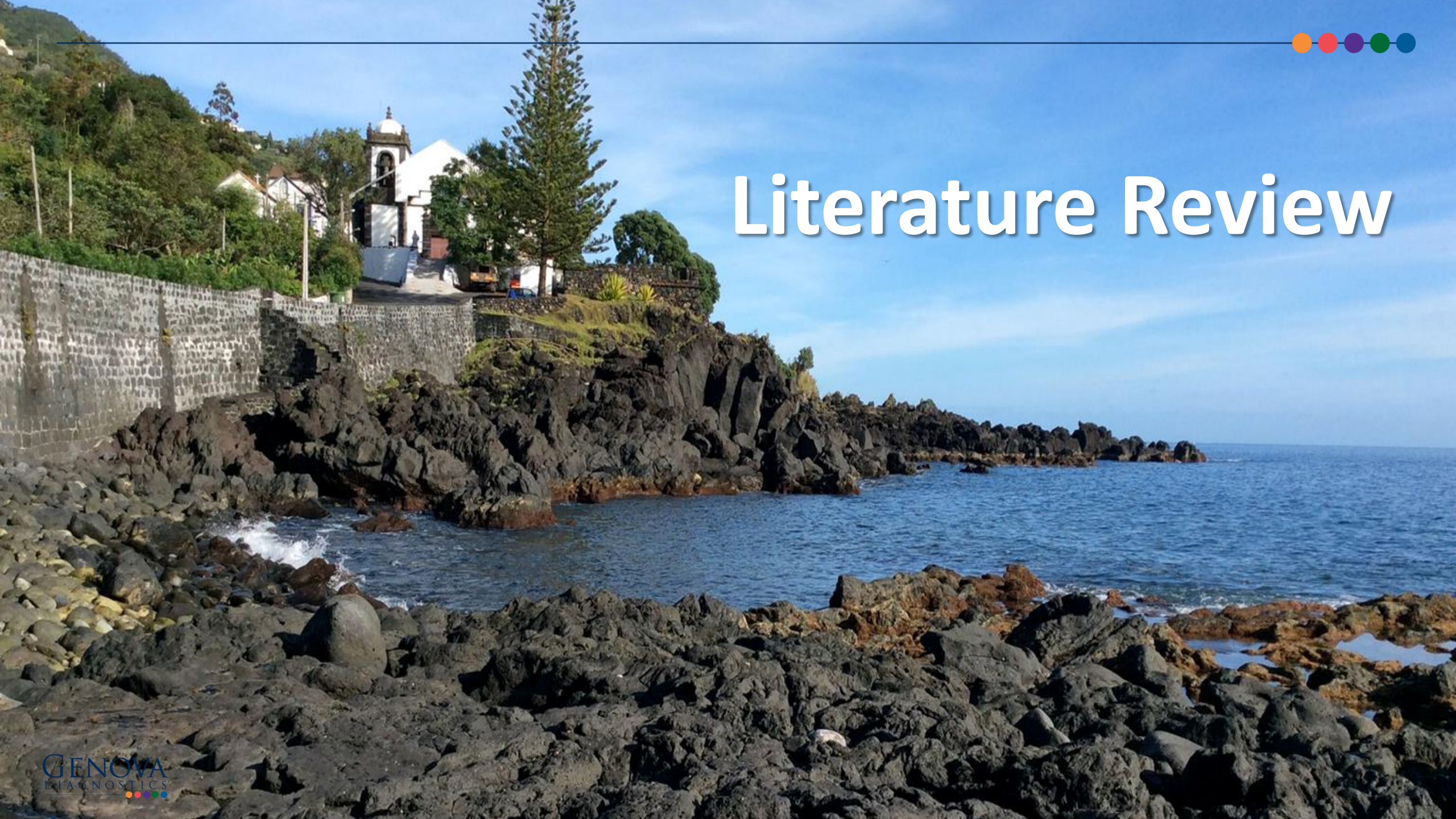

Literature Review

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Carcinogenesis vol.18 no.9 pp.1859–1860, 1997

LETTER TO THE EDITOR

Re: Feigelson,H.S. and Henderson,B.E. (1996) Estrogens and breast cancer. *Carcinogenesis*, 17, 2279–2284

P.David Josephy

University of Guelph, College o
Department of Chemistry and B
Canada N1G 2W1

"Exposure to genotoxic environmental chemicals is a risk factor for breast cancer."

Dear Sir

In a recent issue of the journal, Feigelson and Henderson have presented a thorough review of the relationship between estrogen exposure and breast cancer (1). Certainly, many of the know risk factors for breast cancer, such as early menarche and hormone replacement therapy, can be explained on the basis of hormonal effects. However, the authors state that '*all* [emphasis added] of these [risk factors and protective factors] can be understood as measures of the cumulative exposure of the breast to estrogen and, perhaps progesterone'. This assertion could be interpreted as ruling out a role for genotoxic chemical carcinogens in the etiology of breast cancer.

Mammary carcinogenesis begins with the proliferation of a ductal cell, carrying either germ-line or somatic mutations in critical genes. Hormones provide the proliferative stimulus necessary for progression of these initiated ductal cells to form tumours. Considerable evidence now indicates that exposure to genotoxic environmental chemicals is a risk factor for breast cancer (2).

[illegible] human mammary carcinogenesis'.

In the search for the environmental causes of breast cancer (14), we should not overlook the possible importance of genotoxic carcinogens, as well as hormonal factors.

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Environment International 99 (2017) 107–119

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review article

Environmental impact of estrogens on human, animal and plant life: A critical review

Muhammad Adeel [a], Xiaoming Song [a], Yuanyuan Wang [a], Dennis Francis [a], Yuesuo Yang [a,b,*]

Keywords:
Estrogens
Environmental fate
Endocrine disrupting chemical (EDC)
Plant uptake
Bioavailability
Aquatic ecology
Water and soil

entry of estrogens into the human food chain which in turn relates to how plants take up and metabolism estrogens.
Objectives: In this review we explore the environmental fate of estrogens highlighting their release through effluent sources, their uptake, partitioning and physiological effects in the ecological system. We draw attention to the potential risk of intensive modern agriculture and waste disposal systems on estrogen release and their effects on human health. We also highlight their uptake and metabolism in plants.
Methods: We use MEDLINE and other search data bases for estrogens in the environment from 2005 to the present, with the majority of our sources spanning the past five years. Published acceptable daily intake of estrogens (μg/L) and predicted no effect concentrations (μg/L) are listed from published sources and used as thresholds to discuss reported levels of estrogens in the aquatic and terrestrial environments. Global levels of estrogens from river sources and from Waste Water Treatment Facilities have been mapped, together with transport pathways of estrogens in plants.
Results: Estrogens at polluting levels have been detected at sites close to waste water treatment facilities and in groundwater at various sites globally. Estrogens at pollutant levels have been linked with breast cancer in women and prostate cancer in men. Estrogens also perturb fish physiology and can affect reproductive development in both domestic and wild animals. Treatment of plants with steroid estrogen hormones or their precursors can affect root and shoot development, flowering and germination. However, estrogens can ameliorate the effects of other environmental stresses on the plant.
Conclusions: There is published evidence to establish a causal relationship between estrogens in the environment and breast cancer. However, there are serious gaps in our knowledge about estrogen levels in the environment and a call is required for a world wide effort to provide more data on many more samples sites. Of the data available, the synthetic estrogen, ethinyl estradiol, is more persistent in the environment than natural estrogens and may be a greater cause for environmental concern. Finally, we believe that there is an urgent requirement for inter-disciplinary studies of estrogens in order to better understand their ecological and environmental impact.

"There is published evidence to establish a causal relationship between estrogens in the environment and breast cancer."

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Environment International Volume 99, February 2017, Pages 107-119

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Review

Estrogen metabolites and breast cancer

Richard J. Santen *, Wei Yue, Ji-Ping Wang

Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia Health Sciences System, PO Box 801416, Aurbach Medical Research Building, Charlottesville, VA 22908-1416, United States

ARTICLE IN

Article history:
Available online 26 Augus

Keywords:
Estrogen
Metabolites
Genotoxic
Breast cancer
Aromatase
Adducts

...this data supports the role of estradiol metabolism as one of the components in the development of experimental breast cancer.

Our studies are based on the hypothesis that both receptor-mediated and genotoxic pathways contribute to breast cancer. We initially demonstrated that MCF-7 breast cancer cells and normal breast tissue in aromatase transfected mice contain the enzymes necessary to convert estradiol to the estradiol DNA adducts. We then utilized a highly reductionist model to separately analyze the effect of estrogen receptor alpha (ER) on tumor formation and the effects of estrogen depletion by castration in ER knock out/Wnt-1 (ERKO/Wnt) transgenic animals to assess the effects of estradiol in the absence of an ER. Estradiol was added back in castrate ERKO/Wnt animals to determine if Koch's postulates could be fulfilled to increase the incidence of cancer with administration of exogenous estradiol. Finally, we assessed the effects of an aromatase inhibitor on tumor incidence in non-castrate, ERKO/Wnt animals.

The studies demonstrated the conversion of estradiol to genotoxic metabolites in breast tissue. In addition, knockout of ERα caused a reduction in incidence of tumor formation and a delay in the occurrence of those that formed. Oophorectomy further reduced the incidence of tumors and delayed their onset whereas estradiol add-back returned the incidence rate to that observed before oophorectomy. The aromatase inhibitor, letrozole, delayed the onset of tumor formation. Taken together, these data support a role for estradiol metabolism as one of the components in the development of experimental breast cancer.

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Chemical Research in Toxicology

Article

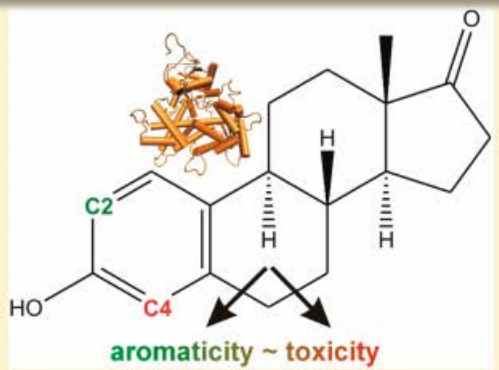
pubs.acs.org/crt

Combined Docking and Quantum Chemical Study on CYP-Mediated Metabolism of Estrogens in Man

Anikó Lábas,[†,§] Balázs Krámos,[†,‡,§] and Julianna Oláh*[,†]

The 4-hydroxylation pathway of estrogens is the most malign and can increase the risk of breast cancer.

ABSTRACT: Long-term exposure to estrogens seriously increases the incidence of various diseases including breast cancer. Experimental studies indicate that cytochrome P450 (CYP) enzymes catalyze the bioactivation of estrogens to catechols, which can exert their harmful effects via various routes. It has been shown that the 4-hydroxylation pathway of estrogens is the most malign, while 2-hydroxylation is considered a benign pathway. It is also known experimentally that with increasing unsaturation of ring B of estrogens the prevalence of the 4-hydroxylation pathway significantly increases. In this study, we used a combination of structural analysis, docking, and quantum chemical calculations at the B3LYP/6-311+G* level to investigate the factors that influence the regioselectivity of estrogen metabolism in man. We studied the structure of human estrogen metabolizing enzymes (CYP1A1, CYP1A2, CYP1B1, and CYP3A4) in complex with estrone using docking and investigated the susceptibility of estrone, equilin, and equilenin (which only differ in the unsaturation of ring B) to undergo 2- and 4-hydroxylation using several models of CYP enzymes (Compound I, methoxy, and phenoxy radical). We found that even the simplest models could account for the experimental difference between the 2- and 4- hydroxylation pathways and thus might be used for fast screening purposes. We also show that reactivity indices, specifically in this case the radical and nucleophilic condensed Fukui functions, also correctly predict the likeliness of estrogen derivatives to undergo 2- or 4-hydroxylation.



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Chem. Res. Toxicol. 2017, 30, 583−594

Carcinogenesis. 2001 Jun;22(6):905-11.

Analysis of potential biomarkers of estrogen-initiated cancer in the urine of Syrian golden hamsters treated with 4-hydroxyestradiol.

Todorovic R[1], Devanesan P, Higginbotham S, Zhao J, Gross ML, Rogan EG, Cavalieri EL.

Author information

Abstract

Estrone (E1) and 17beta-estradiol (E2) are metabolized to catechol estrogens (CE), which may be oxidized to semiquinones and quinones (CE-Q). CE-Q can react with glutathione (GSH) and DNA, or be reduced to CE. In particular, CE-3,4-Q react with DNA to form depurinating adducts (N7Gua and N3Ade), which are cleaved from DNA to leave behind apurinic sites. We report the determination of 22 estrogen metabolites, conjugates and adducts in the urine of male Syrian golden hamsters treated with 4-hydroxyestradiol (4-OHE2). After initial purification, urine samples were analyzed by HPLC with multichannel electrochemical detection and by capillary HPLC/tandem mass spectrometry. 4-Hydroxyestrogen-2-cysteine [4-OHE1(E2)-2-Cys] and N-acetylcysteine

[4-OHE1(E2)-2-

1-N7Gua depuri

the administered

depurinating ad

DNA, respective

accounted for a

methoxy CE wer

These results pr

depurinating DN

used as biomark

estrogen-induce

These results provide strong evidence that exposure to 4-OHE1(E2) leads to the formation of DNA adducts. This process is a putative tumor initiating event.

The estrogen metabolites, conjugates and adducts can be used as biomarkers for detecting susceptibility to estrogen-induced cancer.

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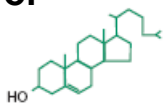
Carcinogeneis, 2001 Jun;22(6):905-11.

ESTROGEN METABOLISM



Cholesterol

Pregnenolone → 17-OH-Pregnenolone → DHEA → Androstenediol

Progesterone → 17-OH-Progesterone → Androstenedione ↔ Testosterone → DHT

(Androgens)

Corticosterone

Cortisol ↔ Cortisone

(Glucocorticoids)

Aldosterone

(Mineralo-corticoids)

Estrone (E1) ↔ Estradiol (E2)

2-OHE1 → 2-MeOE1

16α-OHE1 → Estriol (E3)

4-OHE1 → 4-MeOE1

(Estrogens)

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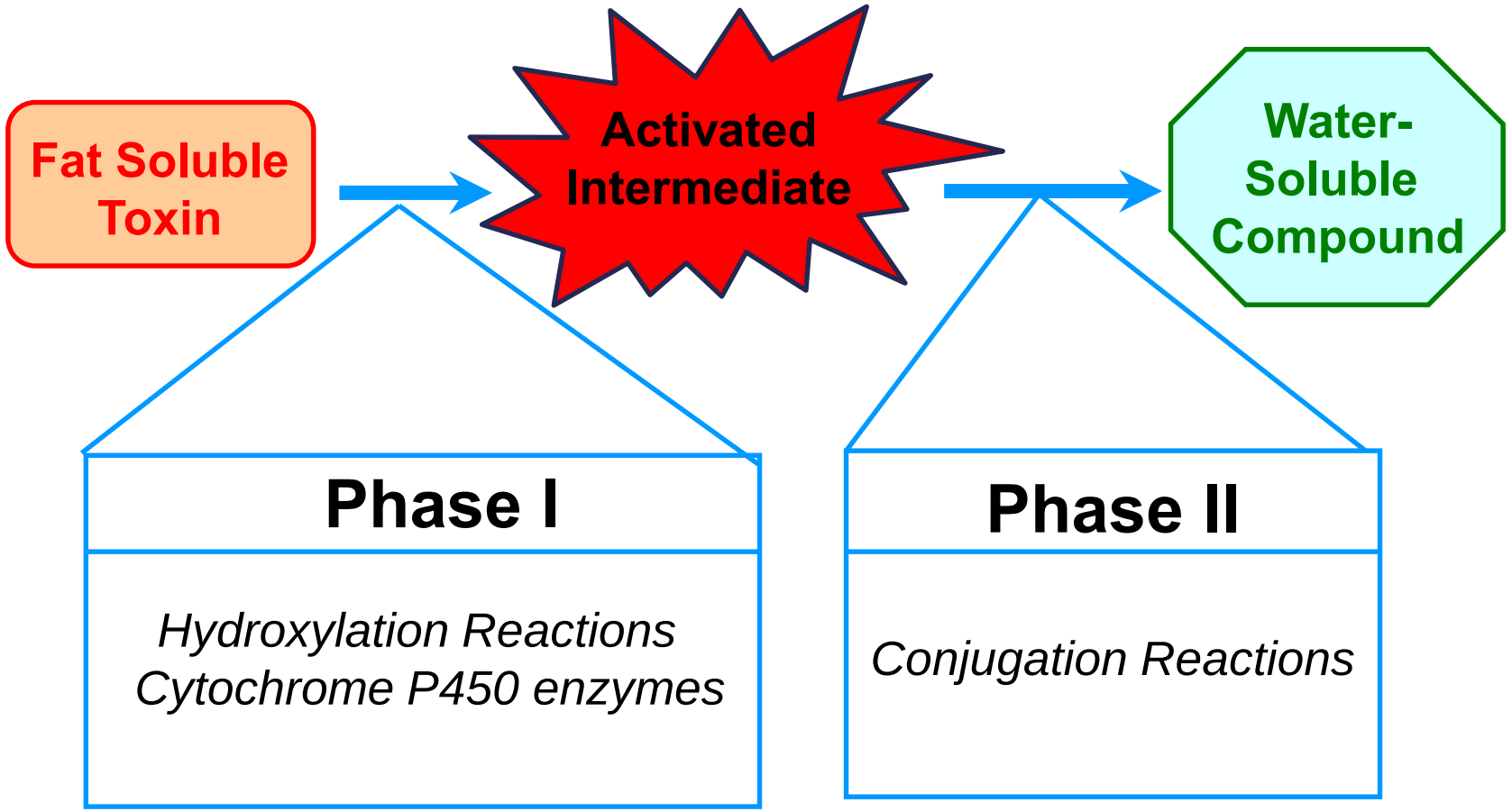
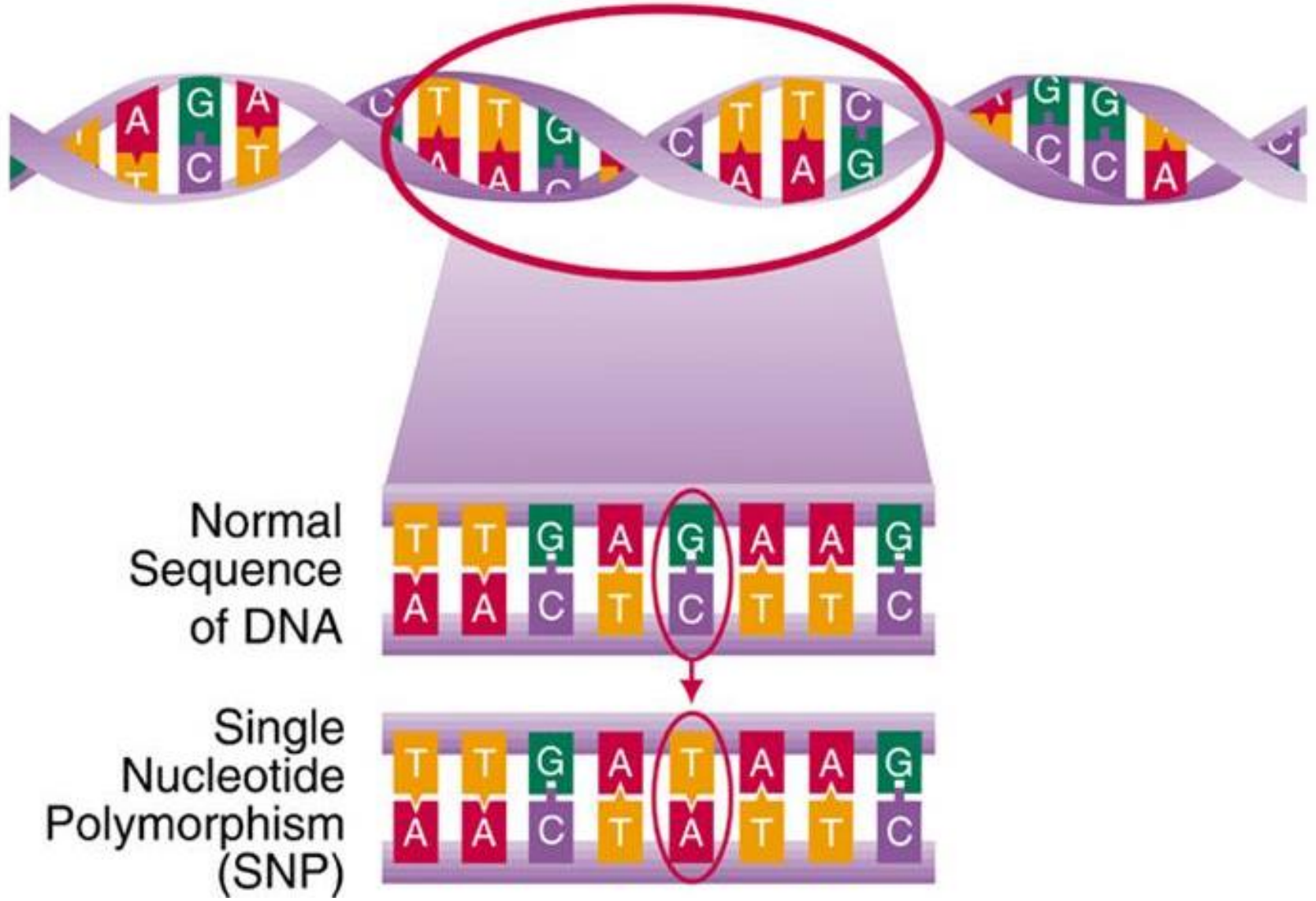
Two Major Pathways of Detoxification



Single Nucleotide Polymorphism (SNP)



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The Metabolism of Estrogen

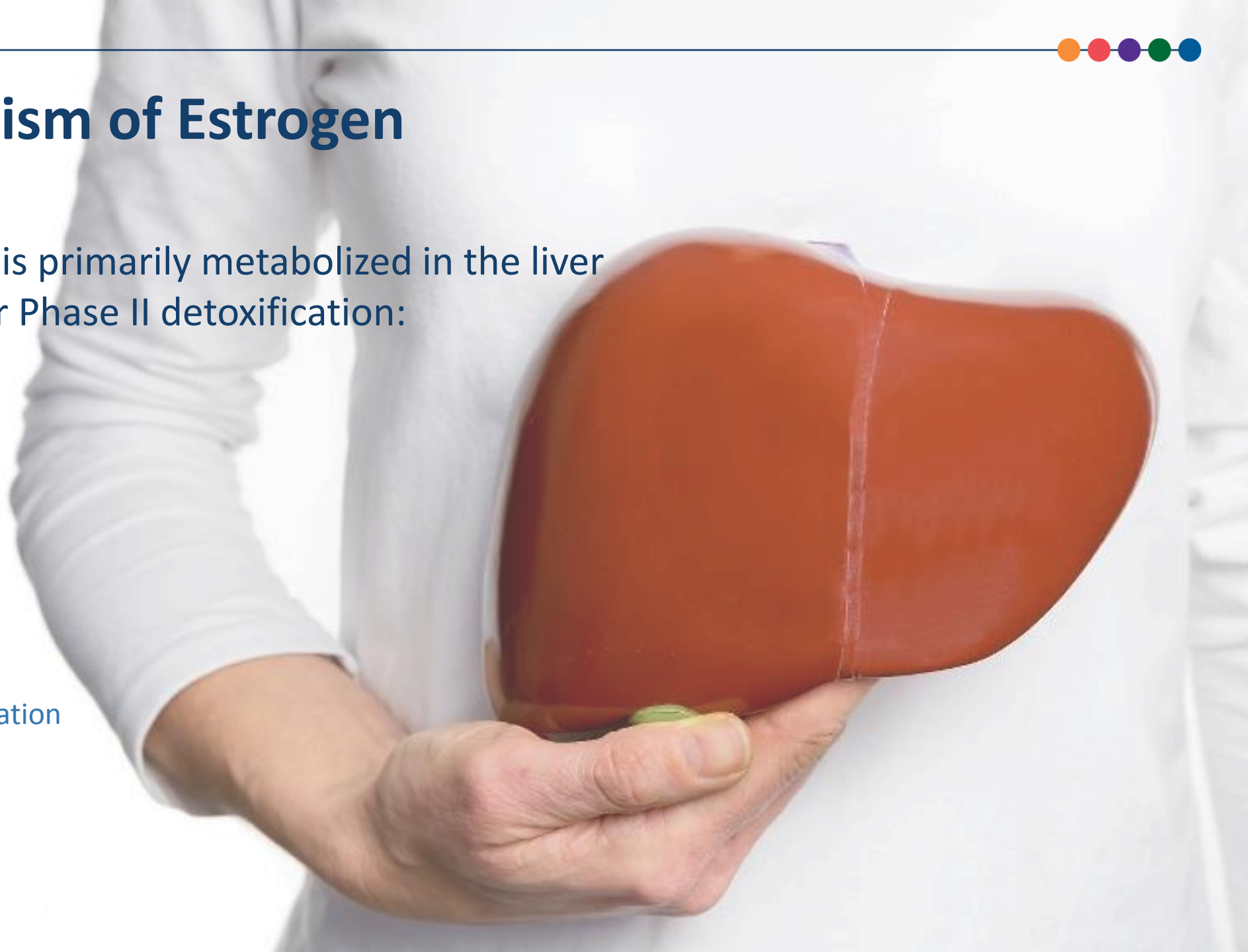
- Unused Estrogen is primarily metabolized in the liver via Phase I and/or Phase II detoxification:

Phase I

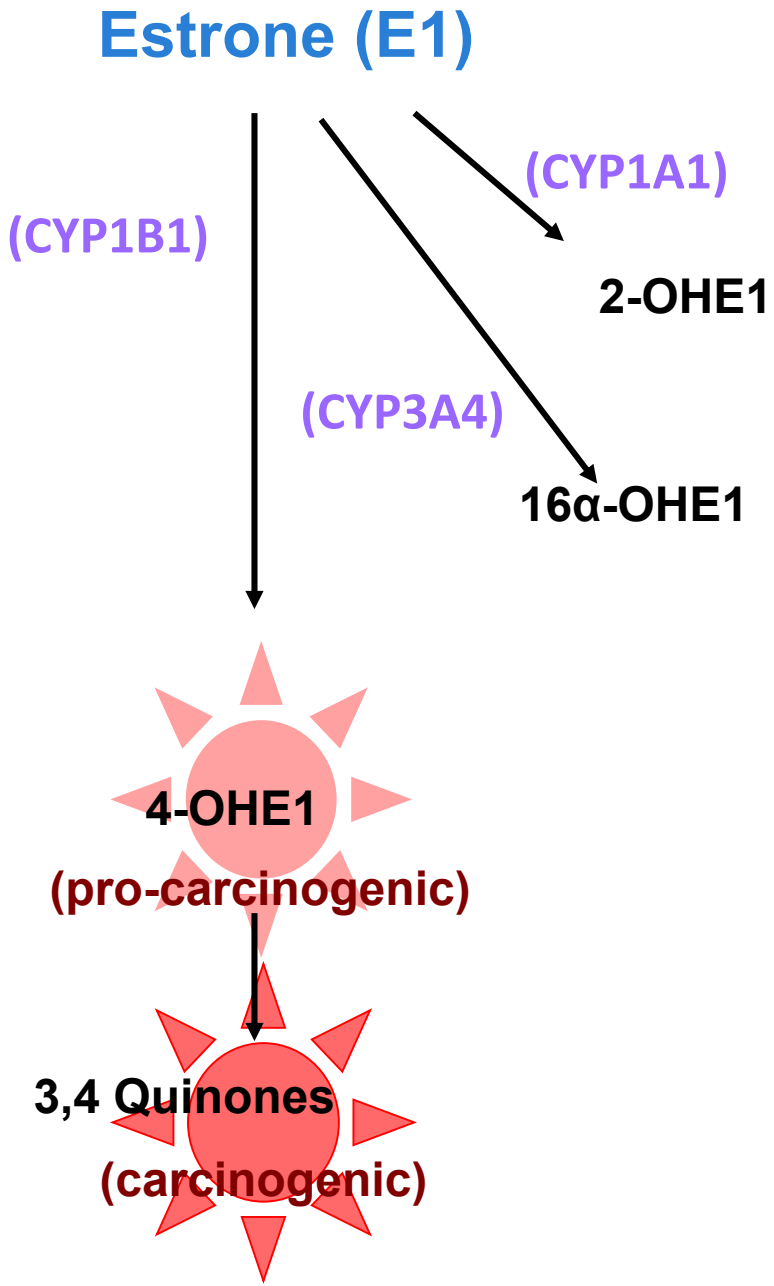
- Major Pathway
- Hydroxylation

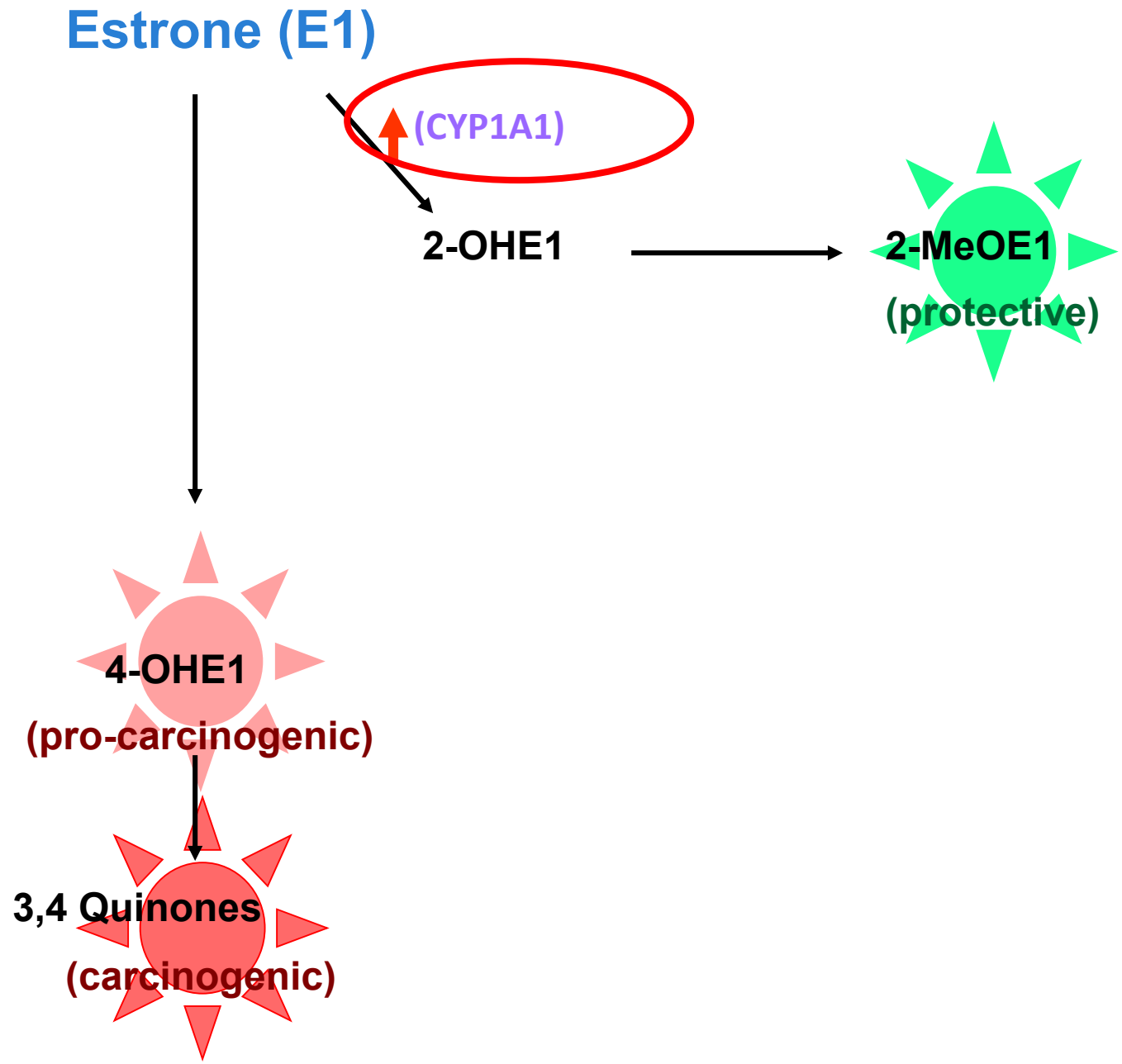
Phase II

- Glucuronidation
- Sulfation
- Methylation
- Glutathione conjugation
- Acetylation

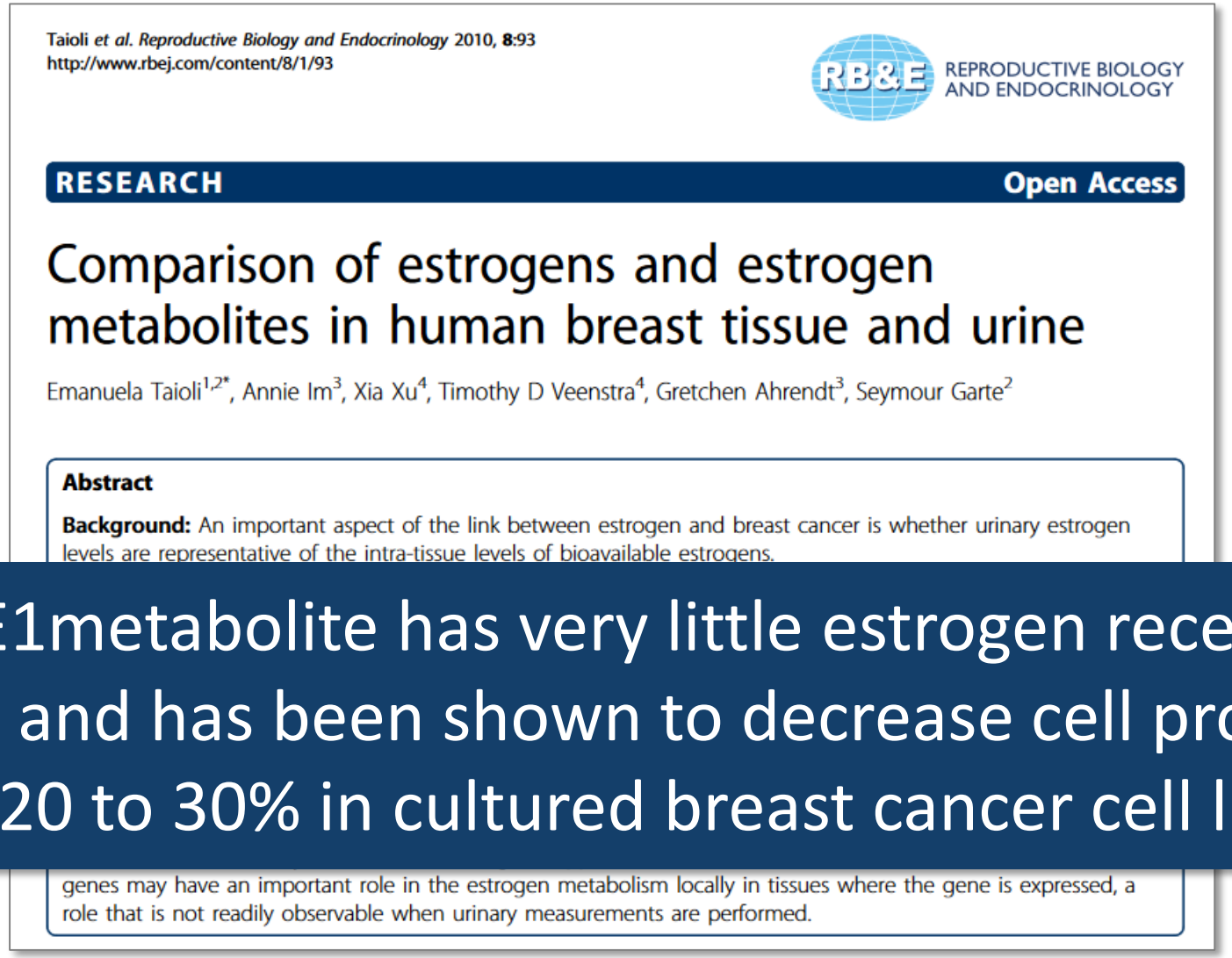


Phase 1 Detoxification


Estrone (E1)
(CYP1B1)
(CYP1A1)
2-OHE1
(CYP3A4)
16α-OHE1
4-OHE1
(pro-carcinogenic)
3,4 Quinones
(carcinogenic)


Estrone (E1)
(CYP1A1)
2-OHE1
2-MeOE1
(protective)
4-OHE1
(pro-carcinogenic)
3,4 Quinones
(carcinogenic)

2-Hydroxyestrone (2-OHE1)



Taioli *et al. Reproductive Biology and Endocrinology* 2010, **8**:93
http://www.rbej.com/content/8/1/93

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH **Open Access**

Comparison of estrogens and estrogen metabolites in human breast tissue and urine

Emanuela Taioli[1,2*], Annie Im[3], Xia Xu[4], Timothy D Veenstra[4], Gretchen Ahrendt[3], Seymour Garte[2]

Abstract

Background: An important aspect of the link between estrogen and breast cancer is whether urinary estrogen levels are representative of the intra-tissue levels of bioavailable estrogens.

genes may have an important role in the estrogen metabolism locally in tissues where the gene is expressed, a role that is not readily observable when urinary measurements are performed.

"2-OHE1metabolite has very little estrogen receptor binding affinity, and has been shown to decrease cell proliferation by 20 to 30% in cultured breast cancer cell lines."

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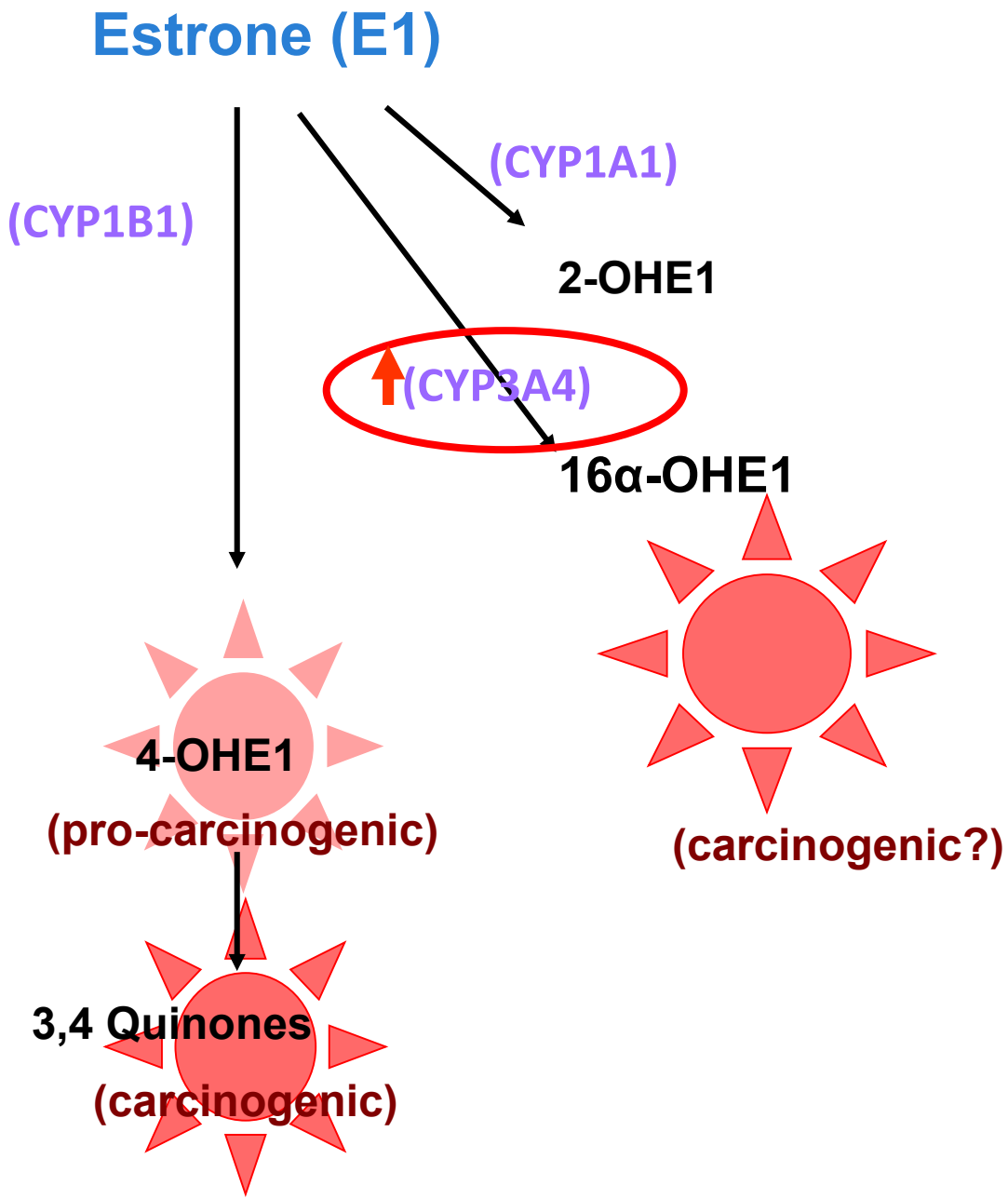
Taioli et al. Reproductive Biology and Endocrinology. 2010, 8:93.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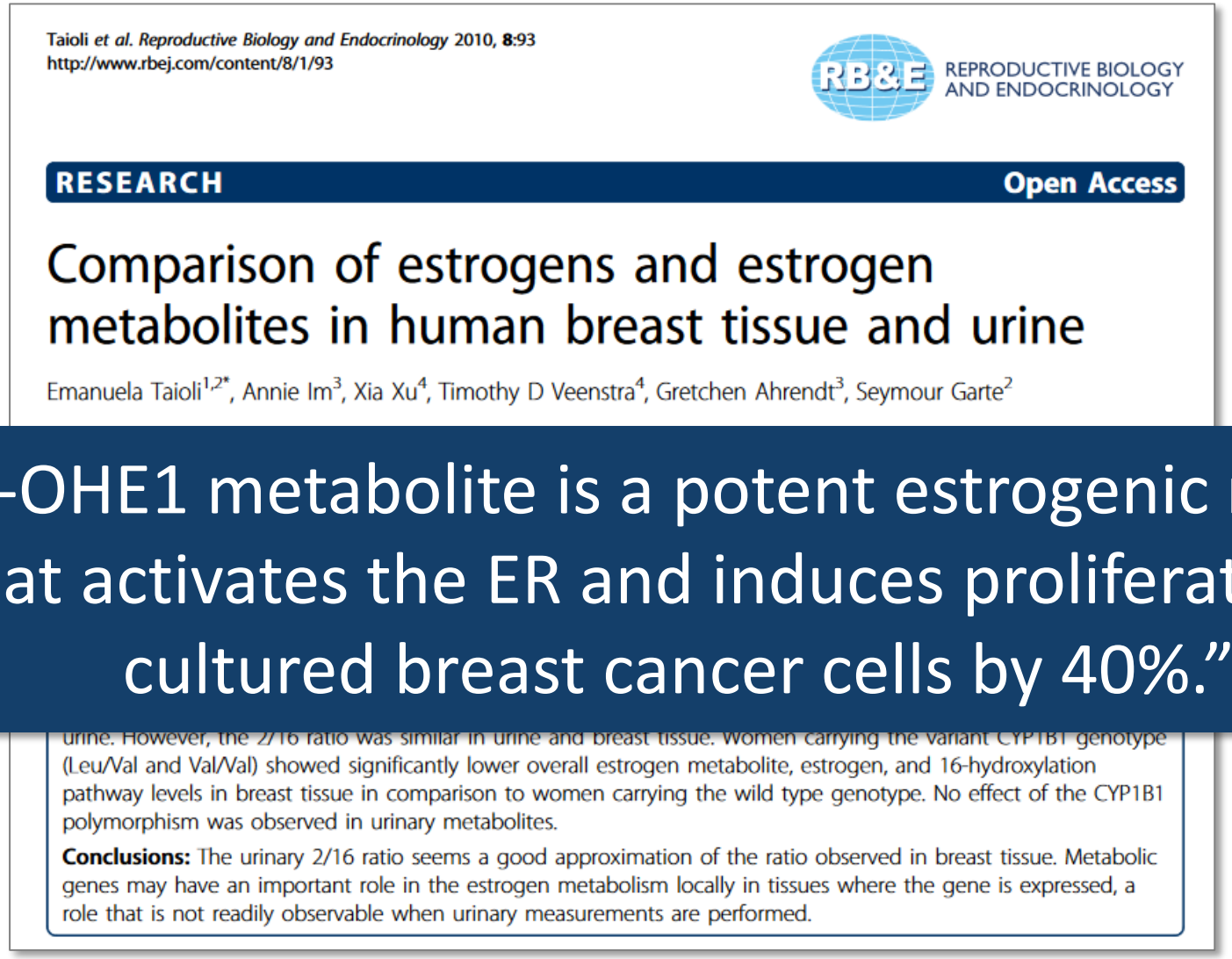
(CYP1B1)
(CYP1A1)
2-OHE1
(CYP3A4)
16α-OHE1
4-OHE1
(pro-carcinogenic)
(carcinogenic?)
3,4 Quinones
(carcinogenic)

16α-Hydroxyestrone (16α-OHE1)

- Strong estrogenic activity
- Turns on estrogen receptor
- Greater likelihood of estrogen-dependent conditions

16α-Hydroxyestrone (16α-OHE1)



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Comparison of estrogens and estrogen metabolites in human breast tissue and urine

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urine. However, the 2/16 ratio was similar in urine and breast tissue. Women carrying the variant CYP1B1 genotype (Leu/Val and Val/Val) showed significantly lower overall estrogen metabolite, estrogen, and 16-hydroxylation pathway levels in breast tissue in comparison to women carrying the wild type genotype. No effect of the CYP1B1 polymorphism was observed in urinary metabolites.

Conclusions: The urinary 2/16 ratio seems a good approximation of the ratio observed in breast tissue. Metabolic genes may have an important role in the estrogen metabolism locally in tissues where the gene is expressed, a role that is not readily observable when urinary measurements are performed.

"16a-OHE1 metabolite is a potent estrogenic molecule that activates the ER and induces proliferation of cultured breast cancer cells by 40%."

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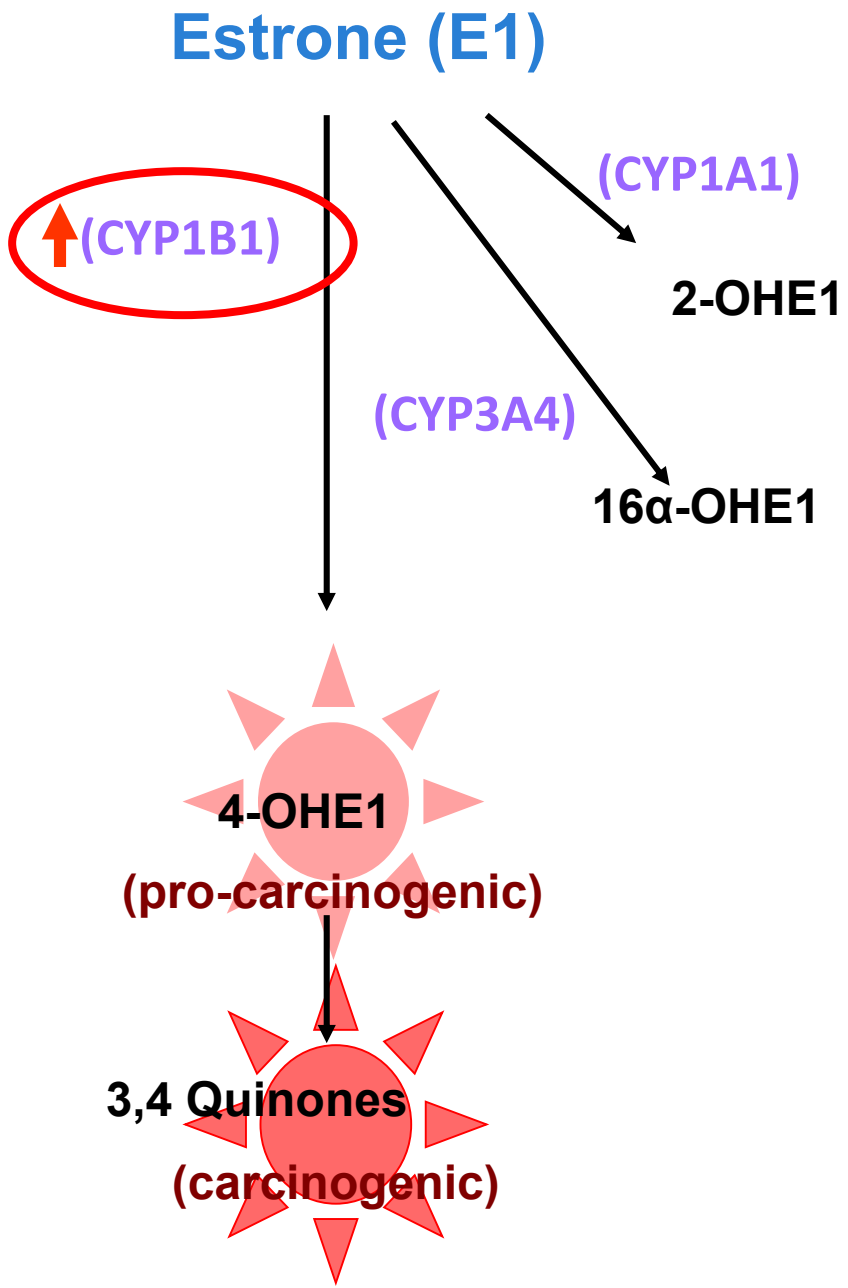
Taioli et al. Reproductive Biology and Endocrinology. 2010, 8:93.

2:16α-OHE1 Ratio - Historic

- Women with breast CA at all ages show increased 16α-hydroxylation
  - (Zumoff B.Obstet Gynecol Colin North Am 1994;21(4):751-2)
- Post-menopausal women at baseline who went on to develop breast CA showed 15% lower 2:16-OHE1 ratio than controls
  - (Meilahn EN 1998. Br J Cancer 1998;78(9):1250-5)
- Association with breast cancer stage: women with lower ratios may have a poorer prognosis
  - (Kabat GC, Cancer Epidemiol Biomarkers Prev 1997;6:505-509)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4-OHE1
(pro-carcinogenic)
3,4 Quinones
(carcinogenic)

Biochim Biophys Acta. 2006 Aug;1766(1):63-78. Epub 2006 Apr 19.

Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention.

Cavalieri E[1], Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, Sutter T.

Author information

Abstract

Exposure to estrogens is associated with increased risk of breast and other types of human cancer. Estrogens are converted to metabolites, particularly the catechol estrogen-3,4-quinones (CE-3,4-Q), that can react with DNA to form depurinating adducts. These adducts are released from DNA to generate apurinic sites. Error-prone base excision repair of this damage may lead to the mutations that can initiate breast, prostate and other types of cancer. The reaction of CE-3,4-Q with DNA forms the depurinating adducts 4-hydroxyestrone(estradiol)-1-N3Ade and 4-OHE1(E2)-1-N7Gua. These two adducts constitute more than 99% of the total DNA adducts form
unbalanced. Such an i
deactivating (protective
cancer, compared to c
prostate cancer patient
perspectives: one is mu
gene in mouse skin an
with the CE-3,4-Q prec
gland within 6-12 h afte
apurinic sites generate
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presence of the antiest
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tumors in estrogen rec
estrogens may cause breast cancer through a genotoxic, non-estrogen receptor-alpha-mediated mechanism. In summary, this evidence strongly indicates that estrogens can become endogenous tumor initiators when CE-3,4-Q react with DNA to form specific depurinating adducts. Initiated cells may be promoted by a number of processes, including hormone receptor stimulated proliferation. These results lay the groundwork for assessing risk and preventing disease.

"In summary, this evidence strongly indicates that estrogens can become endogenous tumor initiators when CE-3,4-Q react with DNA to form specific depurinating adducts."

"Initiated cells may be promoted by a number of processes…. including hormone receptor stimulated proliferation. These results lay the groundwork for assessing risk and preventing disease."

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Carcinogenesis. 2001 Jun;22(6):905-11.

Cytochrome P450 1B1 (CYP1B1)

- Polymorphism is associated with FASTER enzyme activity
- Increased production of 4-OH-estrogens and other potentially carcinogenic compounds
- Tendency for lower 2:16αOH-estrone
- Increased risk of breast cancer, especially if xenobiotic exposure (e.g., PAHs), high BMI, equine estrogens, coexisting CYP1A1 SNP

(Han W 2004, Saintot M 2004, Kocabas NA 2002, Rylander-Rudqvist 2003)

Cytochrome P450 1B1 (CYP1B1)

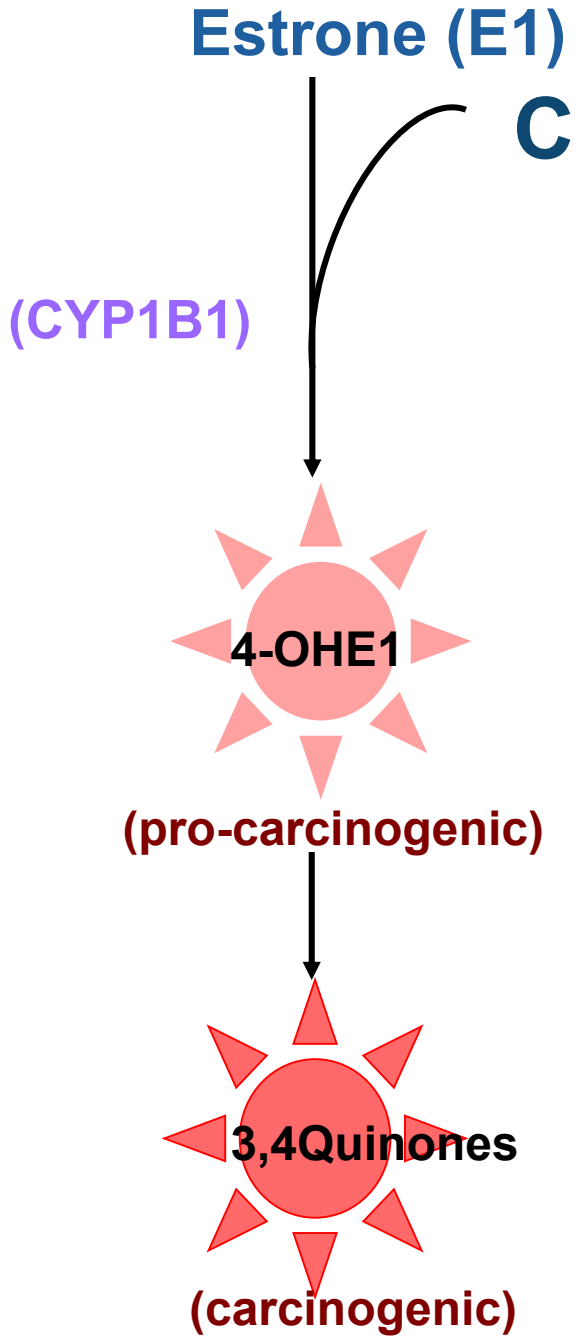
"CYP1B1, which has specific estrogen-4-hydroxylase activity, is present in tissues such as uterus, breast, ovary, and prostate, which often give rise to hormone-responsive cancers"

J Steroid Biochem Mol Biol 2003;86:477–86
Short Review of Pathophysiology of Catechol Estrogen. **Pak J Physiol 2010;6(2)**

4-Hydroxyestrone (4-OHE1)

- Very potent
- If not inactivated by COMT, 4-OHE1 can be oxidized to quinone compounds → DNA adduct formation in tissues such as breast
- Increased 4-hydroxylation of estrogen in uterine fibroids
  - (Reddy VV 1981)
- Link between CYP1B1 SNP (increased 4-OH-estrogen production) and prostate CA
  - (Tang YM 2000)

Conjugated Equine Estrogens



CEEs are preferentially 4-hydroxylated

(Chang M 1998)

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Endocrine Disruptors

- Environmental xenobiotics act as “endocrine disruptors” that modify intercellular communication and function
- Chemicals commonly detected in people include DDT, Polychlorinated biphenyls (PCB's), Bisphenol A (BPA), Polybrominated diphenyl ethers (PBDE's)
- Perfluorinated chemicals (PFC’s)
- May play role in cancer, obesity
- Changes in DNA methylation (epigenetic modification) which can ultimately change ER activity
- Produce a higher ratio of the 4 and 16 hydroxylated estrogen derivatives that are potentially more genotoxic by modifying members of the CYP450 enzyme family

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Latini et al., Mini-Reviews in Medicinal Chemistry, 2010, 10, 846-855
Soto, A. M. & Sonnenschein, C. Nat. Rev. Endocrinol. 6, 363–370 (2010

Hypothesis

Is Bisphenol A a Weak Carcinogen like the Natural Estrogens and Diethylstilbestrol?

Ercole L. Cavalieri[1,2] **and Eleanor G. Rogan**[1,2]

[1]*Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medic*
986805 Nebraska Medical Center, Omaha, NE
[2]*Department of Environmental, Agricultural and Occupational Health, College of Public He*
University of Nebraska Medical Center, 985110 Nebraska Medical Center, Omaha, NE

Summary

Bisphenol A (BPA) displays weak estrogenic properties and could be a weak carcinogen by a mechanism similar to that of estrone (E_1), estradiol (E_2) and the synthetic estrogen diethylstilbestrol, a human carcinogen. A wide variety of scientific evidence supports the hypothesis that certain estrogen metabolites, predominantly catechol estrogen-3,4-quinones, react with DNA to cause mutations that can lead to the initiation of cancer. One of the major pathways of estrogen metabolism leads to the 4-catechol estrogens, 4-OHE_1(E_2), which are oxidized to their quinones, E_1(E_2)-3,4-Q. The quinones react with DNA to form predominantly the depurinating adducts 4-OHE_1(E_2)-1-N3Ade and 4-OHE_1(E_2)-1-N7Gua. This process constitutes the predominant pathway in the initiation of cancer by estrogens. One pathway of BPA metabolism is hydroxylation of one of its symmetric benzene rings to form its catechol, 3-OHBPA. Subsequent oxidation to BPA-3,4-quinone would lead to reaction with DNA to form predominantly the depurinating adducts 3-OHBPA-6-N3Ade and 3-OHBPA-6-N7Gua. The resulting apurinic sites in the DNA could generate mutations in critical genes that can initiate human cancers. The catechol of BPA may also alter expression of estrogen-activating and deactivating enzymes, and/or compete with methoxylation of 4-OHE_1(E_2) by catechol-*O*-methyltransferase, thereby unbalancing the metabolism of estrogens to increase formation of E_1(E_2)-3,4-Q and the depurinating estrogen-DNA adducts leading to cancer initiation. Thus, exposure to BPA could increase the risk of developing cancer by direct and/or indirect mechanisms. Knowledge of these mechanisms would allow us to begin to understand how BPA may act as a weak carcinogen and would be useful for regulating its use.

IUBMB *Life*, 62(10): 746–751, 2010

Keywords estrogens; bisphenc
nism of cancer init
nones.

Bisphenol A (BPA) and
thesized in the 1930's as pot
gens. BPA, however, displaye
It was rediscovered by poly
building block of polycarbon
of epoxy resins and other pla
The worldwide production
pounds per year, and more th
released into the atmosphere
taminate food by leaching f
heated or gets old. Adverse
perimental animals exposed t
detected in 93% of 2517 urin
sample of adults (*2*), indicati
sive throughout the U.S. popu

BPA could be a weak carc
tion similar to that of the natu
carcinogen diethylstilbestrol (
on the basic principles of ch
by James and Elizabeth Mille
on estrogen metabolism (*9–15*), formation of DNA adducts (*12–21*), mutagenicity (*3, 22–25*), cell transformation (*26–29*) and carcinogenicity (*30–33*) led to and support the hypothesis that reaction of specific estrogen metabolites, mostly E_1(E_2)-3,4-qui-

"BPA displays weak estrogenic properties and could be a weak carcinogen by a mechanism similar to that of estrone (E(1)), estradiol (E(2)) and the synthetic estrogen diethylstilbestrol, a human carcinogen…. The catechol of BPA may alter expression of estrogen-activating and deactivating enzymes, and/or compete with methoxylation of 4-OHE(1)(E(2)) by catechol-O-methyltransferase, thereby unbalancing the metabolism of estrogens to increase formation of E(1)(E(2))-3,4-Q and the depurinating estrogen-DNA adducts leading to cancer initiation. Thus, exposure to BPA could increase the risk of developing cancer by direct and/or indirect mechanisms."

GENOVA DIAGNOSTICS

UBMB Life, 62(10): 746–751, October 2010

Int J Environ Res Public Health. 2012 Aug;9(8):2694-714. doi: 10.3390/ijerph9082694. Epub 2012 Jul 31.

Non-genomic effects of xenoestrogen mixtures.

Viñas R[1], Jeng YJ, Watson CS.

Author information

Abstract

Xenoestrogens (XEs) are chemicals derived from a variety of natural and anthropogenic sources that can interfere with endogenous estrogens by either mimicking or blocking their responses via non-genomic and/or genomic signaling mechanisms. Disruption of estrogens' actions through the less-studied non-genomic pathway can alter such functional end points as cell proliferation, peptide hormone release, catecholamine transport, and apoptosis, among others. Studies of potentially adverse effects due to mixtures and to low doses
non-geno
different p
understar
compone

Xenoestrogens (XEs) are chemicals derived from a variety of natural and anthropogenic sources that can interfere with endogenous estrogens by either mimicking or blocking their responses via non-genomic and/or genomic signaling mechanisms.

GENOVA DIAGNOSTICS

J Steroid Biochem Mol Biol. 2011 Oct;127(1-2):44-50. doi: 10.1016/j.jsbmb.2011.01.015. Epub 2011 Feb 12.

Endocrine disruption via estrogen receptors that participate in nongenomic signaling pathways.

Watson CS[1], Jeng YJ, Guptarak J.

Author information

Abstract

When inappropriate (non-physiologic) estrogens affect organisms at critical times of estrogen sensitivity, disruption of normal endocrine functions can result. Non-physiologic estrogen mimetics (environmental, dietary, and pharmaceutical) can signal rapidly and potently via the membrane versions of estrogen receptors, as can physiologic estrogens. Both physiologic and non-physiologic estrogens activate multiple signaling pathways, leading to altered cellular functions (e.g. peptide release, cell proliferation or death, transport). Xenoestrogens' mimicry of physiologic estrogens is imperfect. When superimposed, xenoestrogens can alter endogenous estrogens' signaling and thereby disrupt normal signaling pathways, leading to malfunctions in many tissue types. Though these xenoestrogen actions occur rapi

combinations of ligands, and sig

the activation state of transcripti

stimulation patterns, they must b

xenoestrogens in combination wi

perspectives.

Xenoestrogens can alter endogenous estrogens' signaling and thereby disrupt normal signaling pathways, leading to malfunctions in many tissue types.

GENOVA DIAGNOSTICS

Environ Mol Mutagen. 2014 May;55(4):343-53. doi: 10.1002/em.21847. Epub 2014 Jan 24.

Di-(2-ethylhexyl) phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro.

Zhang T[1], Li L, Qin XS, Zhou Y, Zhang XF, Wang LQ, De Felici M, Chen H, Qin GQ, Shen W.

Author information

Abstract

Bisphenol-A (BPA) and diethylhexyl phthalate (DEHP) are estrogenic compounds widely used in commercial plastic products. Previous studies have shown that exposure to such compounds have adverse effects on various aspects of mammalian reproduction including folliculogenesis. The objective of this study was to examine the effects of BPA and DEHP exposure on primordial follicle formation. We found that germ cell nest breakdown and primordial follicle assembly were significantly reduced when newborn mouse ovaries were exposed to 10 or 100 μM BPA and DEHP in vitro. [illegible] and the mRNA level of the pro-apoptotic gene Bax [illegible] specific genes such as LIM homeobox 8 (Lhx8), fa[illegible] (Sohlh2), and newborn ovary homeobox (Nobox). [illegible] sites of the Lhx8 gene in oocytes, a process norm[illegible] BPA and DEHP exposed ovaries after transplantat[illegible] exposures impair mouse primordial follicle assemb[illegible]

"Finally, folliculogenesis was severely impaired in BPA and DEHP exposed ovaries after transplantation into the kidney capsules of immunodeficient mice.

In conclusion, BPA and DEHP exposures impair mouse primordial follicle assembly in vitro."

GENOVA DIAGNOSTICS

Environ Mol Mutagen. 2014 May;55(4):343-53.

Published in final edited form as:
Steroids. 2007 February ; 72(2): 124–134.

Xenoestrogens are potent activators of nongenomic estrogenic responses

Cheryl S. Watson, **Nataliya N. Bulayeva**, **Ann L. Wozniak**, and **Rebecca A. Alyea**
Biochemistry & Molecular Biology Dept., University of Texas Medical Branch, Galveston TX 77555-0645

Abstract

Studies of the nuclear transcriptiona
explained their actions in mediating
concentrations widespread in the en
their ability to participate in the plet
the last several years. Here we revie
recent evidence that xenoestrogens
pathways culminating in functional
various classes of xenoestrogens (di
endosulfan, and dieldrin) act via a m
and can provoke Ca^{++} influx via L-t
hormones and mimetics can also cau
(ERKs). However, individual estroge
activations compared to each other a
endocrine functions when acting in
assays allow comparison of these ou
alternative signaling pathway usage
estrogenic or antiestrogenic potential of different types of xenoestrogens, and help us to develop strategies for preventing xenoestrogenic disruption of estrogen action in many tissues.

Many xenoestrogens originally deemed “weak” appear to be potent via some nongenomic signaling pathways, and could contribute to these compounds’ ability to disrupt endocrine functions.

Combinations of Physiologic Estrogens with Xenoestrogens Alter ERK Phosphorylation Profiles in Rat Pituitary Cells

Yow-Jiun Jeng and Cheryl S. Watson

Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas, USA

BACKGROUND: Estrogens are potent nongenomic phospho-activators of extracellular-signal–regulated kinases (ERKs). A major concern about the toxicity of xenoestrogens (XEs) is potential alteration of responses to physiologic estrogens when XEs are present simultaneously.

OBJECTIVES: We examined estrogen-induced ERK activation, comparing the abilities of structurally related XEs (alkylphenols and bisphenol A) to alter ERK responses induced by physiologic concentrations (1 nM) of estradiol (E_2), estrone (E_1), and estriol (

METHODS: We quantified hormone/mimetic-induced ER
rat pituitary cell line using a plate immunoassay, compari
and by estrogen receptor subtype-selective ligands.

RESULTS: Alone, these structurally related XEs activate
similar (but not identical) to that with physiologic estroge
lar (active between femtomolar and nanomolar concentrat
estrogen signaling at low, environmentally relevant conce
the lowest, subpicomolar concentrations) and attenuated (
centrations) the actions of the physiologic estrogens. Some
responses/inhibitions. The phosphorylated ERK and pro
ligands were only partially correlated.

CONCLUSIONS: XEs are both imperfect potent estrogens a
cious an XE, the more it disrupts actions of physiologic estrogens. This ability to disrupt physiologic estrogen signaling suggests that XEs may disturb normal functioning at life stages where actions of particular estrogens are important (e.g., development, reproductive cycling, pregnancy, menopause).

KEY WORDS: ERα, ERβ, ERK activation, GPER, membrane estrogen receptors, nongenomic effects, physiologic estrogens, prolactinoma cell line, xenoestrogens. *Environ Health Perspect* 119:104–112 (2011). doi:10.1289/ehp.1002512 [Online 22 September 2010]

traveling down their cascades at varying speeds (Belcheva and Coscia 2002; Bulayeva et al. 2004). Although the less studied physiologic estrogens E_1 and E_3 have weak effects on transcription (Kuiper et al. 1997; Lippman et al. 1977), they are as effective as E_2 in causing

et al. 2002). The alkylphenols are a structurally related (and therefore interesting to compare mechanistically) set of such compounds; they vary in their carbon side-chain length or, in the case of bisphenol A (BPA), have a phenol group instead of an alkyl side chain [see Supplemental Material (doi:10.1289/

Xenoestrogens are both imperfect potent estrogens and endocrine disruptors; the more efficacious a XE, the more it disrupts actions of physiologic estrogens.

GENOVA DIAGNOSTICS

Environ Health Perspect 119:104–112 (2011)

PLoS One. 2014 Feb 20;9(2):e88961. doi: 10.1371/journal.pone.0088961. eCollection 2014.

Xenoestrogens alter estrogen receptor (ER) α intracellular levels.

La Rosa P, Pellegrini M, Totta P, Acconcia F, Marino M.

Author information

Abstract

17β-estradiol (E2)-dependent estrogen receptor (ER) α intracellular concentration is a well recognized critical step in the pleiotropic effects elicited by E2 in several target tissues. Beside E2, a class of synthetic and plant-derive
bind to and modify both nu
is available on the ability o
bisphenol A (BPA) and na
been evaluated on ERα le
phosphorylation and gene
proliferation; whereas 24 h
reported. E2 or BPA treat
stimulation does not alter
experiments indicate that
the physiological ability of
ERα protein accumulation by preventing proteasomal receptor degradation via persistent activation of p38/MAPK pathway. As a whole these data demonstrate that ERα intracellular concentration is an important target through which EDs hamper the hormonal milieu of E2 target cells driving cells to different outcomes or mimicking E2 even in the absence of the hormone.

"These data demonstrate that ERα intracellular concentration is an important target through which EDs hamper the hormonal milieu of E2 target cells driving cells to different outcomes or mimicking E2 even in the absence of the hormone."

GENOVA DIAGNOSTICS

Mol Cell Endocrinol. 2009 May 25;304(1-2):63-8. doi: 10.1016/j.mce.2009.02.016. Epub 2009 Mar 9.

The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes.

Nadal A[1], Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB.

Author information

Abstract

The estrogen receptor ERalph
metabolism. The main functio
only hormone that can directly
exist in beta-cells. The role of
regulation of insulin biosynthes
17beta-estradiol (E2) and the
insulin biosynthesis through a non-classical estrogen-activated pathway that involves phosphorylation of ERK1/2. The activation of ERalpha by physiological concentrations of E2 may play an important role in the adaptation of the endocrine pancreas to pregnancy. However, if ERalpha is over stimulated by an excess of E2 or the action of an environmental estrogen such as BPA, it will produce an excessive insulin signaling. This may provoke insulin resistance in the liver and muscle, as well as beta-cell exhaustion and therefore, it may contribute to the development of type II diabetes.

"If ER alpha is over stimulated by an excess of E2 or the action of an environmental estrogen such as BPA, it will produce an excessive insulin signaling."

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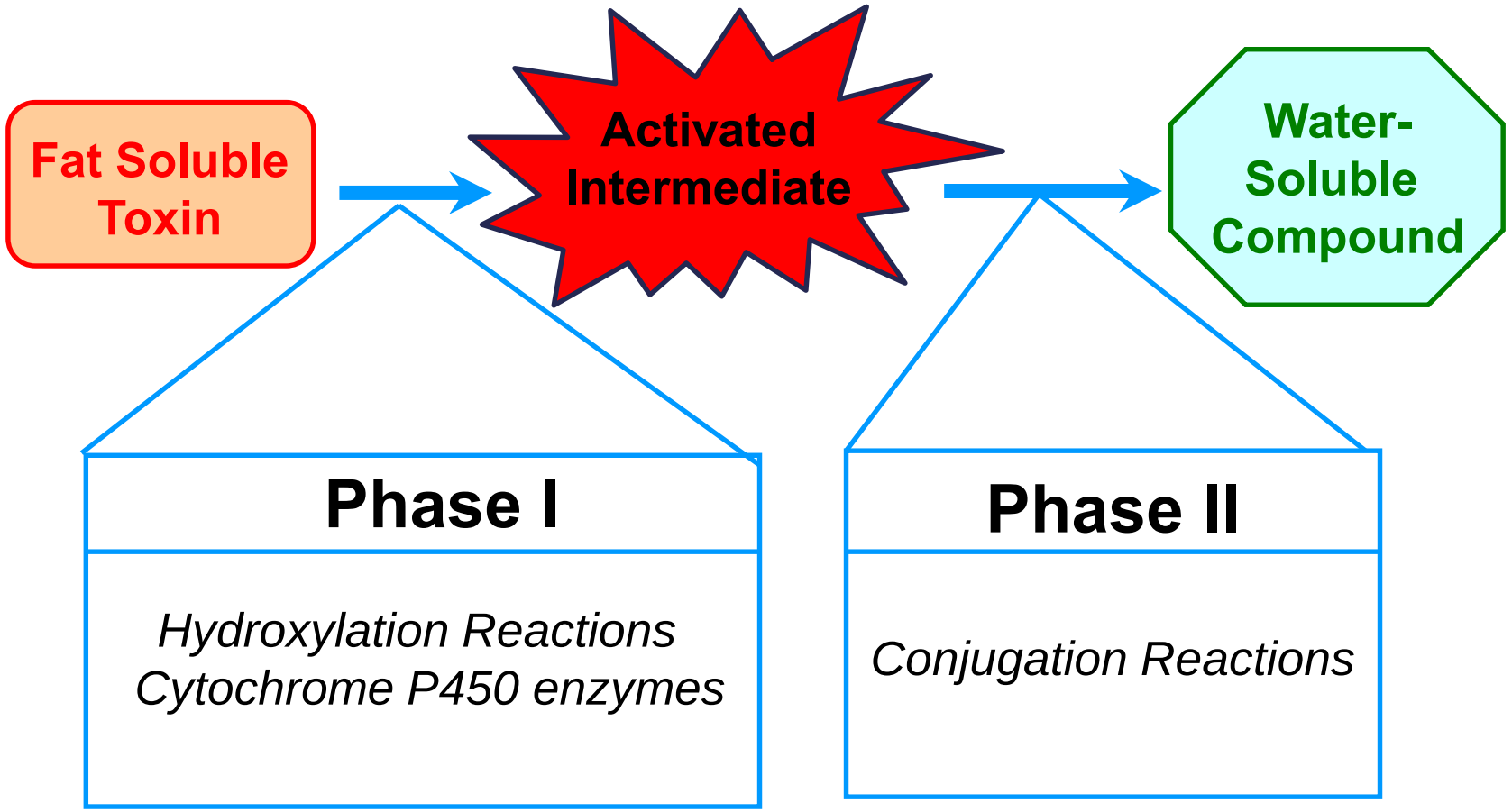
Modulators of CYP450 enzymes

- CYP1A1 and/or CYP1A2:
  - Resveratrol (purple grapes) (blocks dioxin induction via aryl hydrocarbon receptor)
  - Ellagic acid (berries)
  - Green tea catechins (EGCG)
  - Kava
  - DHEA
- CYP1B1:
  - Green tea catechins (EGCG)
  - DHEA
  - Di-indolylmethane
  - I3C
  - Red Clover extracts

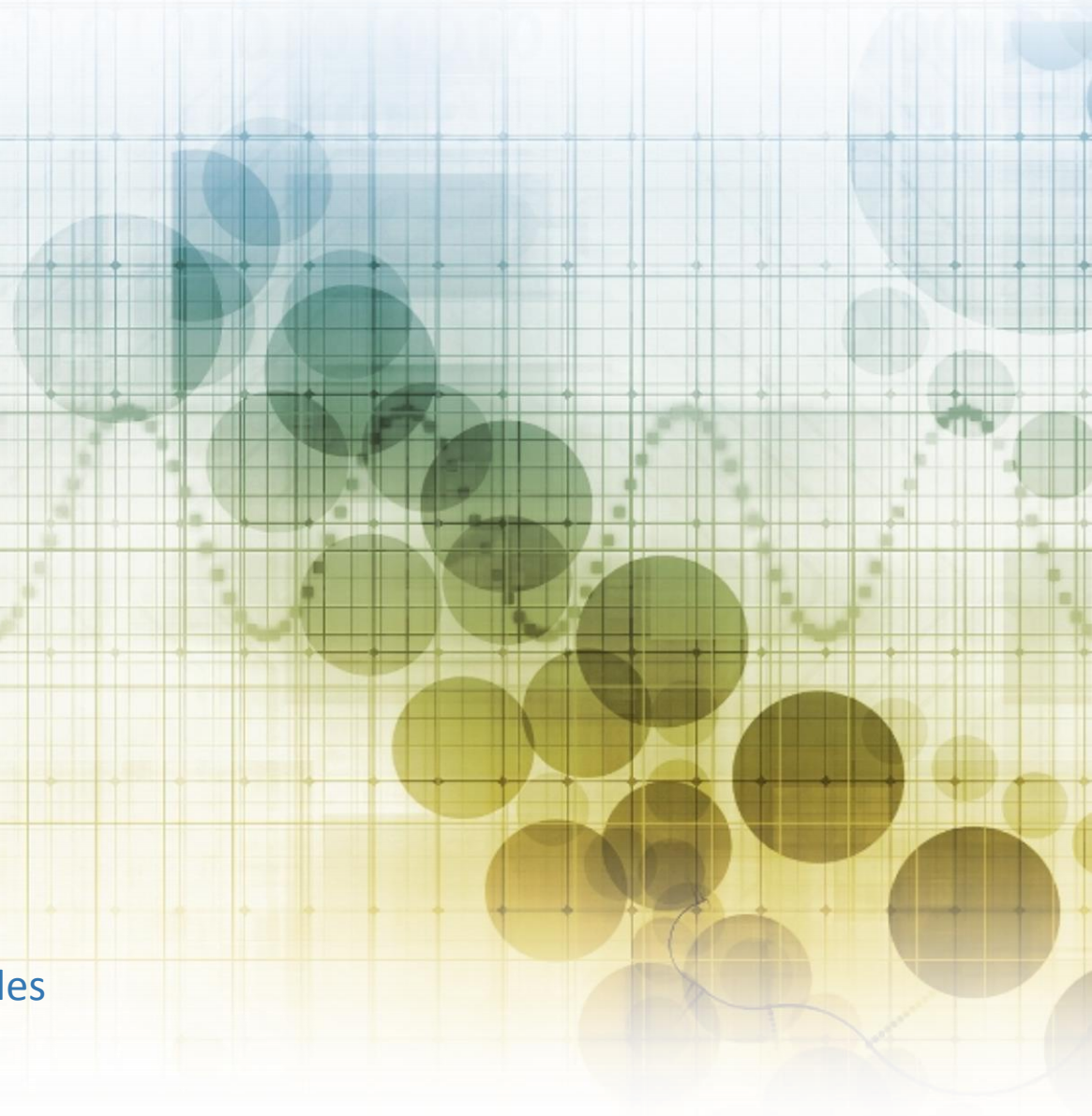
Phase 2 Detoxification

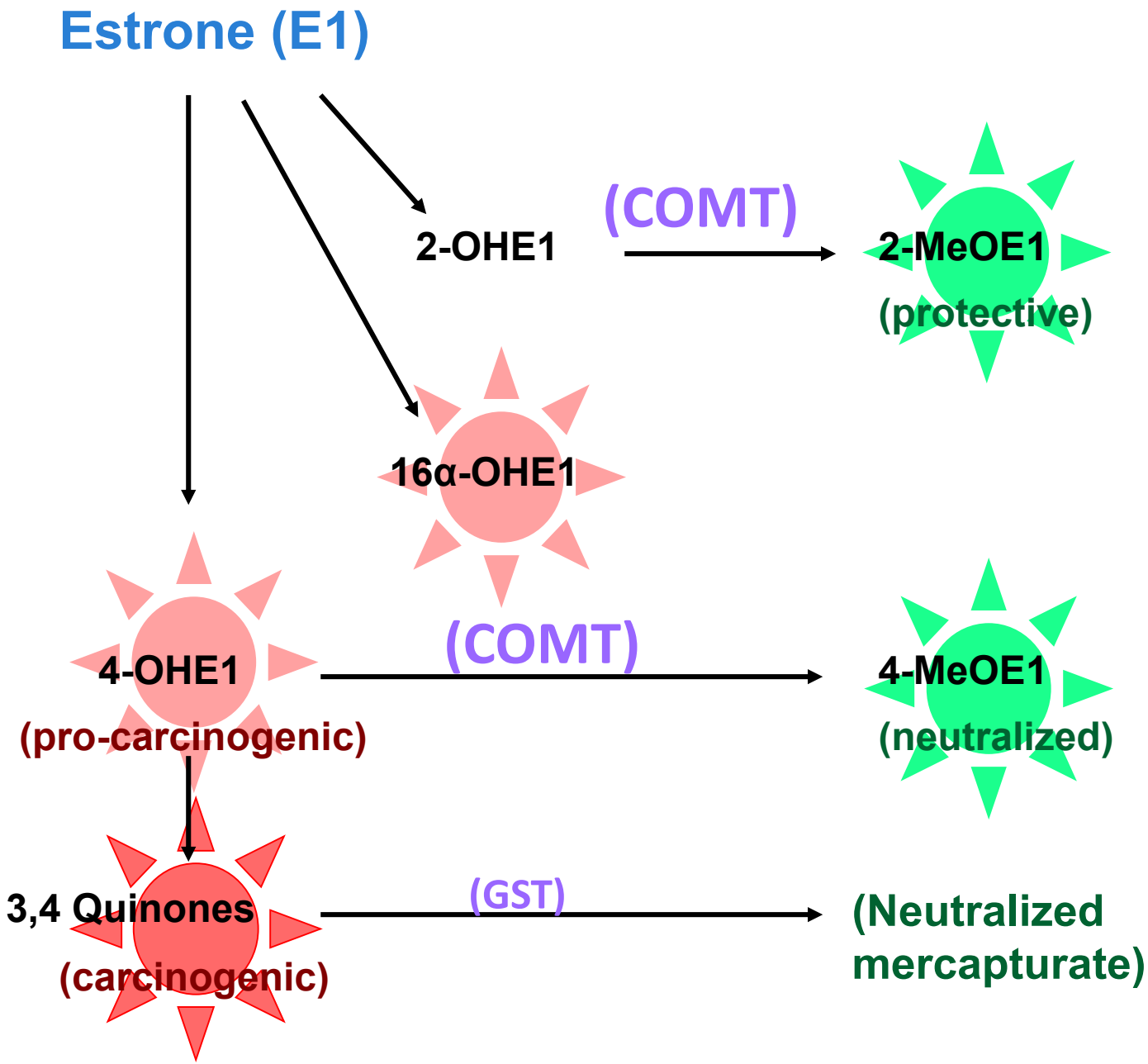
Two Major Pathways of Detoxification



Phase II Substrate Classes

- Sulfation & Glucuronidation
  - Many drugs and xenobiotics (esp. phenolic compounds)
  - Many steroid hormones and the fat-soluble vitamins
  - Bile acids, bilirubin, some neurotransmitters
- Acetylation & Methylation
  - Many drugs and some xenobiotics (esp. metals/minerals)
  - Many neurotransmitters
- Amino Acid Conjugation
  - Some drugs and xenobiotics (esp. aliphatic compounds)
  - Fatty acids and bile acids
- Glutathione Conjugation
  - Few drugs but many xenobiotics (esp. toxic metals)
  - Small carbon molecules, prostaglandins, and lipid peroxides




Estrone (E1)
2-OHE1
(COMT)
2-MeOE1
(protective)
16α-OHE1
4-OHE1
(pro-carcinogenic)
(COMT)
4-MeOE1
(neutralized)
3,4 Quinones
(carcinogenic)
(GST)
(Neutralized
mercapturate)

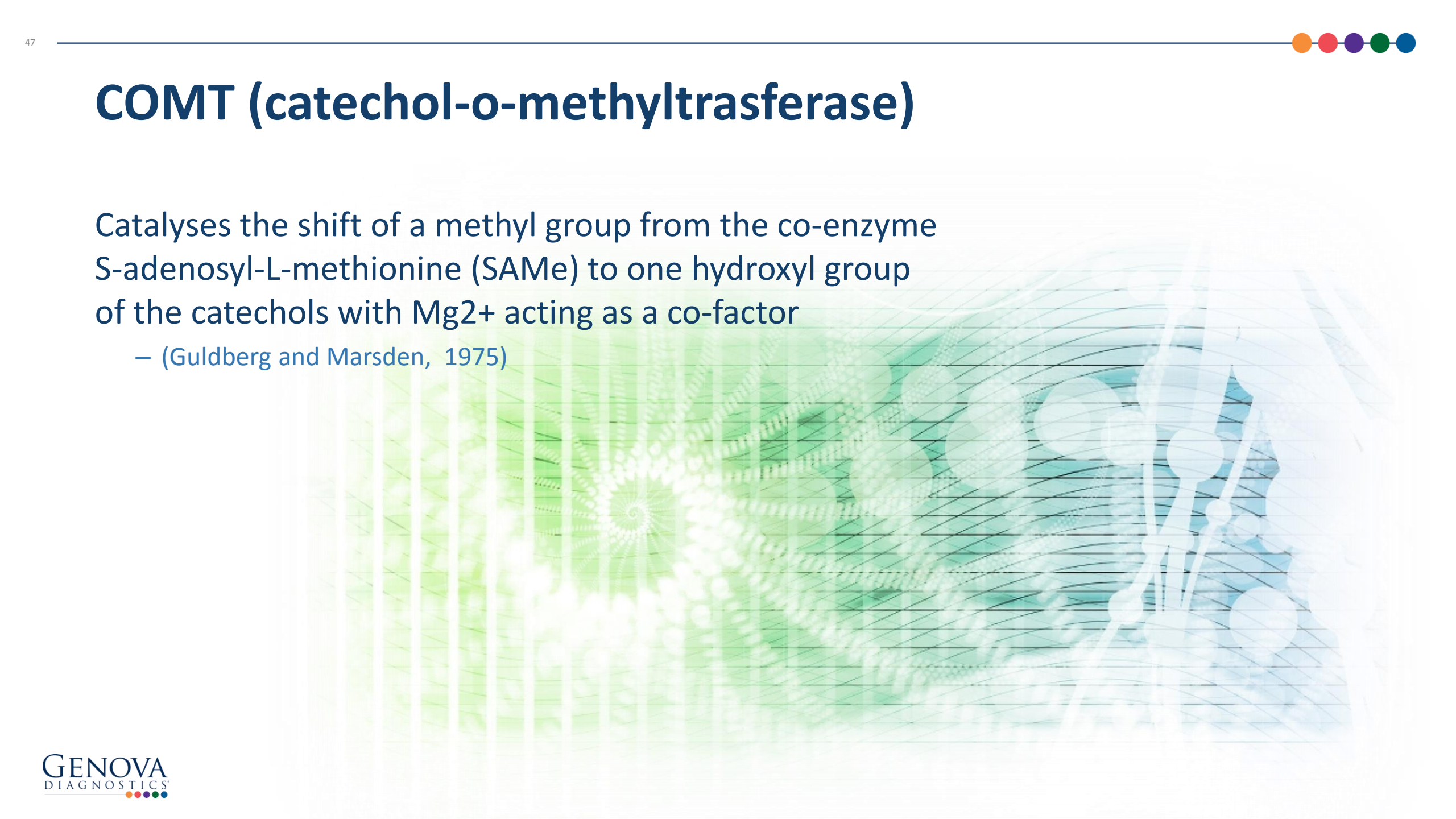
Role of Methylation

- 2-OHE1 is only protective against cancer when methylated by catechol-O-methyltransferase (COMT)
  - 2-methoxy-estrogens are being researched for therapeutic use in breast cancer and CV disease
- 4-OHE1 is less likely to oxidize to carcinogenic compounds if neutralized by COMT
- 2-MeOE1:2-OHE1 and 4-MeOE1:4-OE1 ratios in urine provide a gauge of methylation capacity in a given patient

COMT (catechol-o-methyltrasferase)

Catalyses the shift of a methyl group from the co-enzyme S-adenosyl-L-methionine (SAMe) to one hydroxyl group of the catechols with Mg2+ acting as a co-factor

- (Guldberg and Marsden, 1975)



Catechol-O-methyltransferase (COMT)

- Polymorphism associated with reduced enzyme activity
- Less production of protective 2-methoxy-estrogens, less neutralization of pro-carcinogenic 4-OH-estrogens
- Higher serum levels of E2 in women using E2 HRT
- Impaired clearance of catecholamines → nervousness, anxiety, increased sensitivity to pain

Hum Reprod. 2003 Feb;18(2):262-6

COMT

- Increasing breast CA risk with decreasing COMT activity
- Risk higher in women…
  - With prolonged estrogen exposure
    - (HRT, early menarche, or high BMI) (Huang CS 1999)
  - Low folate or high homocysteine
    - (Goodman JE 2001)
  - Co-existing GST polymorphisms
    - especially if on HRT (Mitrunen K 2002)

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COMT and Breast Cancer

Several epidemiological studies have shown that individuals with low activity form of COMT may have a greater risk for breast cancer.

- Hui Y et al. On the sulfation and methylation of catecholestrogens in human mammary epithelial cells and breast cancer cells. Biol Pharm Bull. 2008 Apr;31(4):769-73.

GENOVA DIAGNOSTICS

Catechol Estrogens and the Uterus

"Catechol estrogens can also stimulate prostaglandin synthesis (Kelly and Abel, 1981) in the uterus, which can influence uterine function such as implantation… But the 2-OH estrogens have no effect on the uterus."

– Juo SH, Wang TN, Lee JN, Wu MT, Long CY, Tsai EM. CYP17, CYP1A1 and COMT polymorphisms and the risk of adenomyosis and endometriosis in Taiwanese women. Hum Reprod. 2006 Jun;21(6):1498-502.

Estrogen Metabolism and Ovarian Cancer

"These findings indicate that estrogen metabolism is unbalanced in ovarian cancer and suggest that formation of estrogen-DNA adducts plays a critical role in the initiation of ovarian cancer."

– Rogan EG, et al. Unbalanced estrogen metabolism in ovarian cancer. Int J Cancer. 2014 May 15;134(10):2414-23.

MTHFR and Breast Cancer Risk

Polymorphisms in genes encoding enzymes of folate metabolism are a focus of breast cancer risk studies due of the role of these enzymes in DNA methylation, synthesis, and repair. Results have been controversial. This case control study showed MTHFR polymorphisms are associated with breast cancer risk when co-existent with methionine synthase (MTR) polymorphisms in the heterozygous state.

GENOVA DIAGNOSTICS

Anticancer Res. 2012 Nov;32(11):4805-11.

Are Urinary Metabolites Representative of Tissue Levels?

"The urinary 2/16 ratio seems a good approximation of the ratio observed in breast tissue."

GENOVA DIAGNOSTICS

Taioli et al. Reproductive Biology and Endocrinology 2010, 8:93

Executive summary

Screening & diagnosis of breast, ovarian & endometrial cancers

- Breast cancer screening by mammography is offered routinely to women over 45–50 years of age, as well as to younger women considered to be at higher risk, but is complicated by overdiagnosis leading to unnecessary diagnostic procedures and treatment.
- The lack of an early screening or a diagnostic method for ovarian cancers often leads to diagnosis at a late stage of the disease, with associated poor survival for these women.
- Early detection of endometrial cancers that have poor prognostic features (e.g., clear cell and serous cancers) is likely to reduce mortality from these cancers.

Clinical sampling & sample collections

- A cancer screening marker has to be validated in a population-based setting and in samples predating diagnosis.

DNA methylation & epigenetic analysis

- Analysis of circulating tumor DNA and its epigenetic modification (methylation) in serum/plasma or vaginal fluid is a novel method for identification of cancer biomarkers.
- Reduced representation bisulfite sequencing offers a cost-effective alternative to whole-genome bisulfite sequencing for the identification of differentially methylated regions in DNA samples.
- In comparison to microarray-based methods, next-generation sequencing enables single nucleotide resolution, analysis of more CpG sites and analysis of samples containing as little as 30–50 ng DNA.
- Digital PCR is a targeted method that provides absolute quantification of a DNA template and can be used for sensitive and specific DNA methylation analysis with an increased signal-to-noise ratio compared with conventional methylation-specific PCR or MethyLight.

Bioinformatics

- Discovering cancer-specific methylated DNA regions requires novel bioinformatic tools to identify the most relevant and most promising regions that show a consistent methylation pattern across all linked CpGs of a particular region in the cancer sample and not in any normal sample (e.g., in white blood cells).

Future perspective

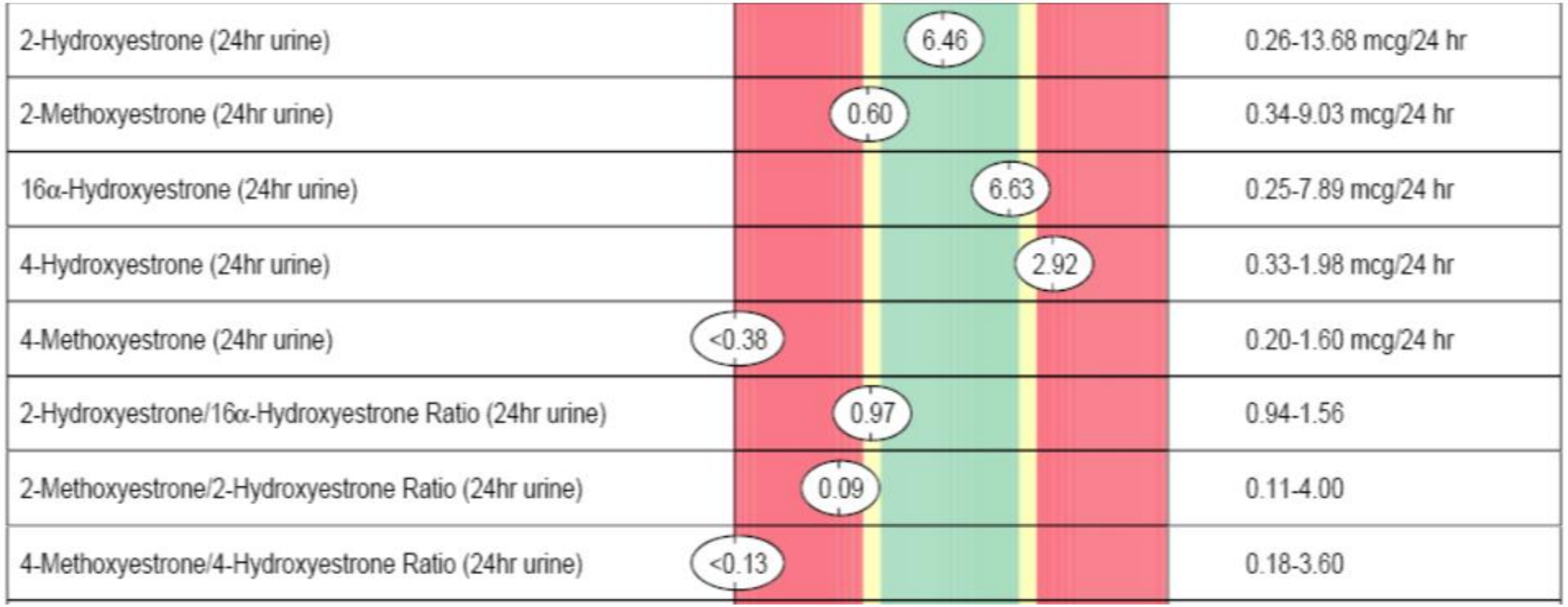
- Aberrant DNA methylation in serum/plasma or vaginal fluid will enable: early identification of individuals before the cancer becomes symptomatic and poses serious risk to well-being; and monitoring and personalization of cancer treatment.

Aberrant DNA methylation in serum/plasma or vaginal fluid will enable early identification of individuals before the cancer becomes symptomatic and poses serious risk to well-being; and monitoring and personalization of cancer treatment.

GENOVA DIAGNOSTICS

Epigenomics (2014) 6(3), 311–327

Estrogen Metabolites



Ann N Y Acad Sci. 2006 Nov;1089:286-301.

Catechol quinones of estrogens in the initiation of breast, prostate, and other human cancers: keynote lecture.

Cavalieri E[1], Rogan E.

Author information

Abstract

Estrogens can be converted to electr
3,4-quinone [E(1)(E(2))-3,4-Q], which
apurinic sites. Error-prone repair of th
reaction of E(1)(E(2))-3,4-Q with DNA
and 4-OHE(1)(E(2))-1-N7guanine(Gu
forms small amounts of the depurinati
abundantly when estrogen metabolism
enzymes and/or deficient expression
imbalance. Oxidation of catechols to s
estrogens, but also for synthetic estro
involved in the initiation of leukemia b
Parkinson's disease by dopamine. In
depurinating N3Ade and N7Gua addu
serve as biomarkers of cancer risk. In
cancer and in women with breast can
diseases suggests preventive strateg
initiation of diseases.

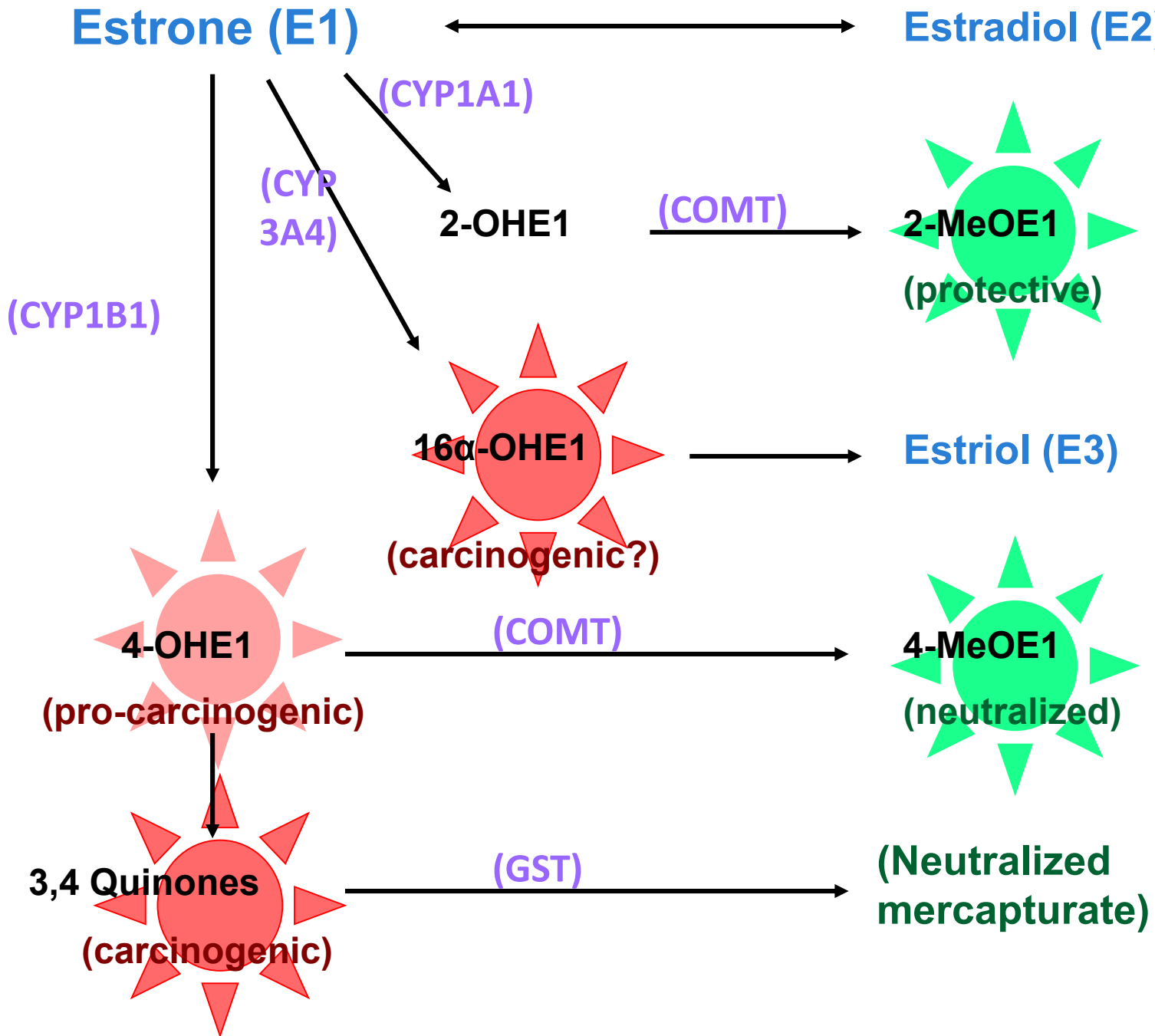
"The depurinating adducts that migrate from cells and can be found in body fluids can also serve as biomarkers of cancer risk.

In fact, a higher level of estrogen-DNA adducts has been found in the urine of men with prostate cancer and in women with breast cancer compared to healthy controls.

This unifying mechanism of the origin of cancer and other diseases suggests preventive strategies based on the level of depurinating DNA adducts that generate the first critical step in the initiation of diseases."

GENOVA DIAGNOSTICS

Ann N Y Acad Sci. 2006 Nov;1089:286-301.



GENOVA DIAGNOSTICS

Carcinogenesis. 2004 Feb;25(2):289-97. Epub 2003 Oct 24.

Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo.

Li KM[1], Todorovic R, Devanesan P, Higginbotham S, Köfeler H, Ramanathan R, Gross ML, Rogan EG, Cavalieri EL.

Author information

Abstract

Studies of estrogen metabolism, formation of DNA adducts, carcinogenicity, cell transformation and mutagenicity have led to the hypothesis that reaction of certain estrogen metabolites, predominantly catechol estrogen-3,4-quinones, with DNA can generate the critical mutations initiating breast, prostate and other cancers. The endogenous estrogens estrone (E1) and estradiol (E2) are oxidized to catechol estrogens (CE), 2- and 4-hydroxylated estrogens, which can be further oxidized to CE quinones. To determine possible DNA adducts of E1(E2)-3,4-quinones [E1(E2)-3,4-Q], we reported previously that the reaction of E1(E2)-3,4-Q with dG

"The most common pathway of conjugation of 4-OHE1(E2) in extrahepatic tissues occurs by O-methylation, which is catalyzed by the ubiquitous catechol-O-methyltransferase (COMT).

This inactivating pathway is in competition with the activation of CE to semiquinones and quinones…. The quinones can be inactivated by formation of glutathione (GSH) conjugates and/or by reduction to CE by quinone reductase.

If, however, these two processes are insufficient, the CE-3,4-quinones can react with DNA to form depurinating adducts (4-OHE1(E2)-1-N3Ade and 4-OHE1(E2)-1-N7Gua)."

GENOVA DIAGNOSTICS

Carcinogenesis. 2004 Feb;25(2):289-97

Phase I

Cytochrome P-450		
Result	Gene	
🔴	CYP1A1 *	www.genovations.com/gdgen01
🔴	CYP1B1 *	www.genovations.com/gdgen02
✔	CYP2A6	www.genovations.com/gdgen10
🔴	CYP2C9 *	www.genovations.com/gdgen05
🔴	CYP2C19 *	www.genovations.com/gdgen06
✔	CYP2D6	www.genovations.com/gdgen03
✔	CYP2E1	www.genovations.com/gdgen04
✔	CYP3A4 *	www.genovations.com/gdgen07

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Phase II

Phase II

Methylation

Result	Gene	SNP Location	Internet Information	Affects
+-	COMT	V158M	www.genovations.com/gdv158m	Liver/Gut

Acetylation (N-acetyl transferase)

SLOW METABOLIZER POLYMORPHISM

Result	Gene	SNP Location	Internet Information	Affects
- -	NAT1	R64W	www.genovations.com/gdr64w	All Cells
- -	NAT1	R187Q	www.genovations.com/gdr187q	Liver/Gut
- -	NAT2	I114T	www.genovations.com/gdi114t	Liver/Gut
+-	NAT2	R197Q	www.genovations.com/gdr197q	Liver/Gut
- -	NAT2	G286E	www.genovations.com/gdg286e	Liver/Gut
- -	NAT2	R64Q	www.genovations.com/gdr64q	Liver/Gut

FAST METABOLIZER POLYMORPHISM

- -	NAT2	K268R	www.genovations.com/gdk268r	Liver/Gut

Glutathione Conjugation (Glutathione s-transferase)

Result	Gene	Location	Internet Information	Affects
ABSENT	GSTM1	1p13.3	www.genovations.com/gdgstm1	Liver/Kidney
- -	GSTP1	I105V	www.genovations.com/gdgstp1	Brain/Skin
- -	GSTP1	A114V	www.genovations.com/gda114v	Brain/Skin

GENOVA DIAGNOSTICS

Phase II Continued

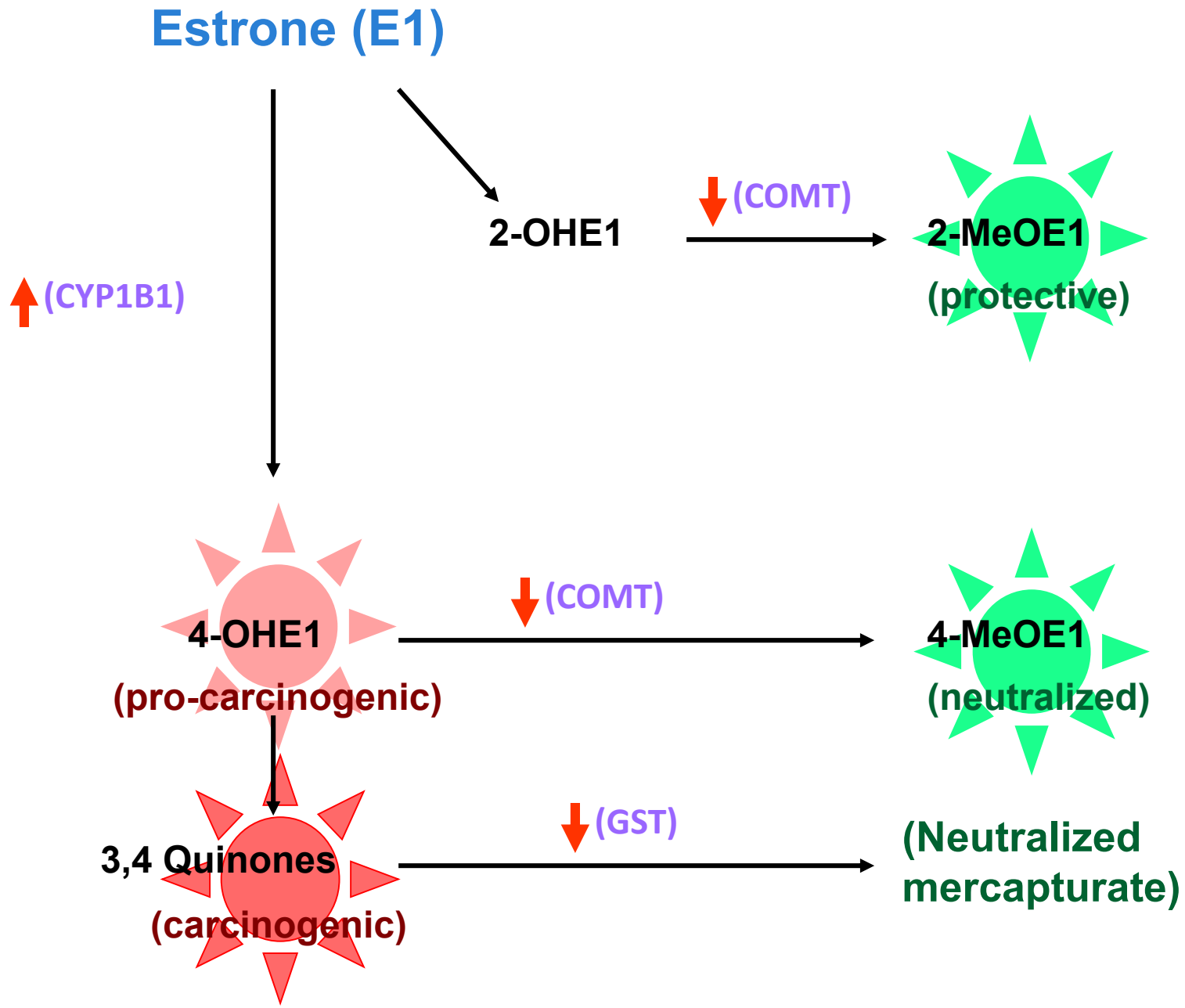
Glutathione Conjugation (Glutathione s-transferase)

Result	Gene	Location		Affects
ABSENT	GSTM1	1p13.3	www.genovations.com/gdgstm1	Liver/Kidney
- -	GSTP1	I105V	www.genovations.com/gdgstp1	Brain/Skin
- -	GSTP1	A114V	www.genovations.com/gda114v	Brain/Skin

Oxidative Protection

Result	Gene	SNP Location	Internet Information	Affects
- -	SOD1	G93A		Cytosol
- -	SOD1	A4V	www.genovations.com/gda4v	Cytosol
- -	SOD2	A16V	www.genovations.com/gda16v	Mitochondria

GENOVA DIAGNOSTICS


Estrone (E1)
2-OHE1
(COMT)
2-MeOE1
(protective)
(CYP1B1)
4-OHE1
(pro-carcinogenic)
(COMT)
4-MeOE1
(neutralized)
3,4 Quinones
(carcinogenic)
(GST)
(Neutralized
mercapturate)


Treatment

Breast Cancer Res Treat. 2010 Jan;119(2):463-74. doi: 10.1007/s10549-009-0407-0. Epub 2009 May 8.

Genetic polymorphisms in phase I and phase II enzymes and breast cancer risk associated with menopausal hormone therapy in postmenopausal women.

MARIE-GENICA Consortium on Genetic Susceptibility for Menopausal Hormone Therapy Related Breast Cancer Risk[1].

Collaborators (30)

Author information

Erratum in

Breast Cancer Res Treat. 2010 Jan;119(2):475.

Abstract

Recent findings indicate a greater risk of postmeno
monotherapy, and more so for current than past use
menopausal hormone therapy. We used two popula
patients and 5,496 controls to evaluate modificatio
in hormone metabolism and detoxification. Twenty-
CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP3A7
SULT1A1, UGT1A1, UGT1A6, UGT2B7) were gen
progestagen therapy was significantly modified by
carriers of the CYP1B1_142_G and the CYP1B1_
confidence interval (CI) = 1.02-1.09) and 1.06 (95%
non-carriers of either polymorphism (p(interaction)
at significantly higher risks associated with hormone use compared with non-carriers (p(interaction) = 0.0001 and 0.02). CYP1A1_2452_C>A significantly reduced the risk associated with duration of use of estrogen monotherapy (p(interaction) = 0.01). The finding regarding GSTT1 was still statistically significant after corrections for multiple comparisons. Postmenopausal breast cancer risk associated with hormone therapy may be modified by genetically determined variations in phase I and II enzymes involved in steroid hormone metabolism.

"Postmenopausal breast cancer risk associated with hormone therapy may be modified by genetically determined variations in phase I and II enzymes involved in steroid hormone metabolism."

GENOVA DIAGNOSTICS

How Do We Decrease Our Risk?

- Proper Risk Assessment
  - Connect the dots
- Decrease exposure
- Promote detoxification
- Enhance Elimination
- Awareness

Cancer Res. 2016 Dec 23. pii: canres.1717.2016. doi: 10.1158/0008-5472.CAN-16-1717. [Epub ahead of print]

Association of Estrogen Metabolism with Breast Cancer Risk in Different Cohorts of Postmenopausal Women.

Sampson JN[1], Falk RT[1], Schairer C[1], Moore SC[1], Fuhrman BJ[2], Dallal CM[3], Bauer DC[4], Dorgan JF[5], Shu XO[6], Zheng W[6], Brinton LA[1], Gail MH[7], Ziegler RG[8], Xu X[9], Hoover R[1], Gierach GL[10].

⊕ Author information

Abstract

Endogenous estradiol and estrone are linked causally to increased risks of breast cancer. In this study, we evaluated multiple competing hypotheses for how metabolism of these parent estrogens may influence risk. Prediagnostic concentrations of estradiol, estrone, and 13 metabolites were measured in 1298 postmenopausal cases of breast cancer and 1524 matched controls in four separate patient cohorts. Median time between sample collection and diagnosis was 4.4-12.7 years across the cohorts. Estrogen analytes were measured in serum or urine by liquid chromatographic-tandem

associated strongly and positively with b

2-hydroxylation pathway metabolites, or

breast cancer risk. These associations v

tertile. With appropriate validation, these

metabolism profiles through either lifesty

PMID: 28011624 DOI: 10.1158/0008-5472.CAN

[PubMed - as supplied by publisher]

"These findings suggest opportunities for breast cancer prevention by modifying individual estrogen metabolism profiles through either lifestyle or chemopreventive strategies."

GENOVA DIAGNOSTICS

Overview of My Protocol

- Decrease Exposures
- Nutritional Support with wholesome food
  - (fresh, whole, unprocessed, organic, colorful, high fiber, with nuts, seeds and omega 3's) herbs, spices, fermented products, soluble fiber and low glycemic load
- Decrease Insulin Stimulation
- Elimination Diet
  - 21-day or longer, personalize
- Targeted Supplementation
  - Food is the foundation
- Lifestyle Modification
- Address digestion
- Exercise/Movement
- Sleep
- Mind-body-spirit connection
- Support

Scents and Perfumes

- Air Fresheners/Deodorizers
- Fabric Softeners
- Scented Candles
- Body Perfumes

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Cosmetic & Personal Care Products

Organic Plant Sources-Toxin Free
Avoid Glandulars
Free of Chemical Colorings
Free of Chemical Preservatives
Free of TALC
Free of Organochlorines
Safe Packaging
Safe Processing Methods

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Toxic Ingredients to Avoid

- DEA (DIETHANOLAMINE), MEA (MONOETHANOLAMINE), TEA (TRIETHANOLAMINE)
- PARABENS PRESERVATIVES (METHYL, PROPYL, BUTYL, ISOBUTYL, and ETHYL)
- MINERAL OIL: PETROLATUM, PETROLEUM JELLY (LIQUID PARAFFINUM, PARAFFIN OIL, PARAFFIN WAX, POSH MINERAL OIL)
- PROPYLENE GLYCOL/BUTYLENE GLYCOL
- SILICONE DERIVED EMOLLIENTS (DIMETHICONE, DIMETHICONE COPOLYOL, CYCLOMETHICONE)
- TALC
- DIBUTYL PHTHALATE
- 1.4–DIOXANE
- BHT (BUTILATED HYDROXYTOLUENE)/BHA(BUTILATED HYDROXYANISOLE)
- BENZALKONIUM CHLORIDE and BENZETHONIUM CHLORIDE
- TRICLOSAN AND TRICLOCARBAN
- PARFUM

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Common Personal Care Products

- Fabric detergents
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- Clothing softeners
  - Rinse cycle
  - Dryer
- Clothing
  - Choose natural fibers

Cosmetic & Personal Care Products

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- Toners
- Make-up
- Gels
- Hair Spray
- Hair Dyes
- Nail Polish

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- **Cabbage**
- **Eggplant**
- **Cantaloupe**
- **Watermelon**
- **Grapefruit**
- **Sweet Potato**
- **Honeydew & Melon**

Int J Cancer. 1998 Mar 16;75(6):825-30.

Breast cancer risk, meat consumption and N-acetyltransferase (NAT2) genetic polymorphisms.

Ambrosone CB[1], Freudenheim JL, Sinha R, Graham S, Marshall JR, Vena JE, Laughlin R, Nemoto T, Shields PG.

Author information

Abstract

Although inconsistencies exist, some studies have sho
Several heterocyclic amines (HAs), formed in the coo
are activated by polymorphic N-acetyltransferase (NA
investigated whether ingestion of meat, chicken and fish, as well as particular concentrated sources of HAs, was associated with breast cancer risk, and if NAT2 genotype modified risk. Caucasian women with incident breast cancer (n = 740) and community controls (n = 810) were interviewed and administered a food frequency questionnaire. A subset of these women (n = 793) provided a blood sample. Polymerase chain reaction and restriction fragment length polymorphism analyses were used to determine NAT2 genotype. Consumption of red meats, as well as an index of concentrated sources of HAs, was not associated with increased breast cancer risk, nor did risk vary by NAT2 genotype. In post-menopausal women, higher fish consumption was inversely associated with risk (odds ratio = 0.7; 95% confidence interval, 0.4-1.0); among pre-menopausal women, there was the suggestion of inverse associations between risk and pork and chicken intake. Our results suggest that consumption of meats and other concentrated sources of HAs is not associated with increased breast cancer risk. However, due to the strong biologic plausibility for a role of some HAs in mammary carcinogenesis, and the likely measurement error in evaluation of sources of HAs in this study, further studies of these possible relationships are warranted.

PMID: 9506525 [PubMed - indexed for MEDLINE]

...higher fish consumption was inversely associated with risk.

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0163-4984/05/10301–0017 $30.00

Quantification and Speciation of Mercury and Selenium in Fish Samples of High Consumption in Spain and Portugal

Ana I. Cabañero,[1] Cristina Carvalho,[2] Yolanda Madrid,[1] Camila Batoréu,[2] and Carmen Cámara[1,*]

Sardines have the best ratio of Selenium/Mercury

ABSTRACT

Mercury (Hg) and selenium (Se) determinations were carried out to evaluate human exposure to those elements through fish consumption in Spain and Portugal. Atomic fluorescence spectroscopy (AFS) was applied in a cold vapor mode for total mercury quantification and was also hyphenated to gas chromatography (GC) to achieve the speciation of organomercurial species in fish samples. The results obtained show the highest concentration of Hg in swordfish and tuna (0.47 ± 0.02 and 0.31 ± 0.01 μg g^{-1}, respectively), and a much lower concentration in sardine, mackerel shad, and octopus (0.048 ± 0.002, 0.033 ± 0.001, and 0.024 ± 0.001 μg g^{-1}, respectively). The determination of alkyl mercury compounds revealed that 93–98% of mercury in the fish samples was in the organic form. Methylmercury (MeHg) was the only species found in the three fish species with higher mercury content.

Total selenium concentration was high in sardine, swordfish, and tuna (0.43 ± 0.02, 0.47 ± 0.02, and 0.92 ± 0.01 μg g^{-1}, respectively), but low in mackerel shad and octopus (0.26 ± 0.01 and 0.13 ± 0.01 μg g^{-1}, respectively). Speciation of selenium compounds was done by high-performance liquid

GENOVA DIAGNOSTICS

Am J Clin Nutr. 2013 Feb;97(2):344-53. doi: 10.3945/ajcn.112.034025. Epub 2012 Dec 26.

Dietary fiber intake and risk of hormonal receptor-defined breast cancer in the European Prospective Investigation into Cancer and Nutrition study.

Ferrari P[1], Rinaldi S, Jenab M, Lukanova A, Olsen A, Tjønneland A, Overvad K, Clavel-Chapelon F, Fagherazzi G, Touillaud M, Kaaks R, von Rüsten A, Boeing H, Trichopoulou A, Lagiou P, Benetou V, Grioni S, Panico S, Masala G, Tumino R, Polidoro S, Bakker MF, van Gils CH, Ros MM, Bueno-de-Mesquita HB, Krum-Hansen S, Engeset D, Skeie G, Pilar A, Sánchez MJ, Buckland G, Ardanaz E, Chirlaque D, Rodriquez L, Travis R, Key T, Khaw KT, Wareham NJ, Sund M, Lenner P, Slimani N, Norat T, Aune D, Riboli E, Romieu I.

Author information

Abstract

BACKGROUND: Lim
(BC) by menopaus

OBJECTIVE: We inv
legumes) and BC ri

DESIGN: A total of 1
follow-up of 11.5 y.
regression models were used to quantify the association between dietary variables and BC risk with energy adjustment by using the residual method. Subgroup analyses were performed by menopausal status and estrogen receptor (ER) and progesterone receptor (PR) expression in tumors.

RESULTS: BC risk was inversely associated with intakes of total dietary fiber [hazard ratio comparing fifth quintile to first quintile (HR(Q5-Q1)): 0.95; 95% CI: 0.89, 1.01; P-trend = 0.03] and fiber from vegetables (0.90; 0.84, 0.96; P-trend < 0.01) but not with fiber from fruit, cereals, or legumes. Overall, associations were homogeneous by menopausal status and ER and PR expression in tumors. For vegetable fiber, stronger associations were observed for estrogen receptor-negative and progesterone receptor-negative (HR(Q5-Q1):0.74; 95% CI: 0.59, 0.93; P-trend = 0.01) than for estrogen receptor-positive and progesterone receptor-positive tumors (0.92: 0.81, 1.03; P-trend = 0.05), with P-heterogeneity = 0.09.

CONCLUSION: Diets rich in dietary fiber and, particularly, fiber from vegetables may be associated with a small reduction in risk of BC, independently of menopausal status.

"Diets rich in dietary fiber and, particularly, fiber from vegetables may be associated with a small reduction in risk of BC, independently of menopausal status."

GENOVA DIAGNOSTICS

Am J Clin Nutr. 2013 Feb;97(2):344-53

Am J Clin Nutr. 2011 Sep;94(3):900-5. doi: 10.3945/ajcn.111.015578. Epub 2011 Jul 20.

Dietary fiber intake and risk of breast cancer: a meta-analysis of prospective cohort studies.

Dong JY[1], He K, Wang P, Qin LQ.

Author information

Abstract

BACKGROUND: Obser
results are inconclusiv

OBJECTIVE: We aimed
meta-analysis of prosp

DESIGN: Relevant stud
were also reviewed. W
intake and breast canc

RESULTS: We identifie
712,195 participants. The combined RR of breast cancer for the highest compared with the lowest dietary fiber intake was 0.89 (95% CI: 0.83, 0.96), and little evidence of heterogeneity was observed. The association between dietary fiber intake and risk of breast cancer did not significantly differ by geographic region, length of follow-up, or menopausal status of the participants. Omission of any single study had little effect on the combined risk estimate. Dose-response analysis showed that every 10-g/d increment in dietary fiber intake was associated with a significant 7% reduction in breast cancer risk. Little evidence of publication bias was found.

CONCLUSION: This meta-analysis provides evidence of a significant inverse dose-response association between dietary fiber intake and breast cancer risk.

A meta-analysis of 10 prospective studies showing a significant inverse association between dietary fiber and risk of BC, with no differences by geographical region or menopausal status.

GENOVA DIAGNOSTICS

Am J Clin Nutr. 2011 Sep;94(3):900-5.

Methylation Support

- COMT uses SAMe as its methyl donor; therefore, maintaining SAMe availability will encourage COMT activity
  - Methionine-esp important if low homocysteine
  - Magnesium
  - B2, B6, B12
  - Folic acid (also as folinic acid, 5-formyl THF, or 5-methyl THF)
  - TMG (betaine)

Dietary Supplements to Optimize Reduced Glutathione Levels

- **Reduced glutathione** 1-3 g/day
- **N-acetyl-cysteine** 600-3,000 mg/day
- **Lipoic acid** 200-1,000 mg/day
- **Whey protein concentrates** 2-3 servings/day
- **Magnesium** 400 + mg
- **Vitamin C** 500+mg
- **Vitamin E** 400 IU
- **Silymarin** 400-1,200 mg/day
- **Pantothenic acid** 500-1,000 mg/day
- **SAMe** 400-800 mg/day

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Dietary Supplements to Optimize Reduced Glutathione Levels Continued…

- Glycine and Glutamine
- B vitamins (B2, B6, B12, 5MTHF)
- Extracts:
  - Turmeric extract, grape seed extract
  - Bilberry, strawberry/black raspberry extracts
- Antioxidants
  - (to discourage formation of quinone compounds)
- Melatonin
- Theanine
- Sulforaphane

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Clinical Cases

Case #1:

CS is 51 yo menopausal woman still menstruating but having increasing hot flashes, night sweats, fatigue and insomnia.

Low estrogen and low progesterone on testing, low thyroid and stage 1 adrenal fatigue.

Started on HRT, thyroid replacement and adaptogens as well as lifestyle modification.

Good symptom control.

Feeling great on follow-up.

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Estrogen Metabolites

		Reference Range
2-Hydroxyestrone (24hr urine)	26.7	9.2-76.6 mcg/g Creat.
16α-Hydroxyestrone (24hr urine)	8.5	2.4-20.3 mcg/g Creat.
4-Hydroxyestrone (24hr urine)	9.1	<= 5.3 mcg/g Creat.
2-Methoxyestrone (24hr urine)	3.2	>= 1.7 mcg/g Creat.
4-Methoxyestrone (24hr urine)	0.7	>= 1.9 mcg/g Creat.

Ratios

Anabolic/Catabolic Balance (24hr urine)	0.6	1.0-3.9
11β-HSD Index (24hr urine)	0.35	0.59-1.42
E/A: 5β/5α Ratio (24hr urine)	0.5	0.8-2.6
2-Hydroxyestrone/16α-Hydroxyestrone Ratio	3.1	1.7-2.8
2-Methoxyestrone/2-Hydroxyestrone Ratio	0.12	>= 0.09

Treament

- Increase cruciferous vegetables
- Methylating factors
- I3C/Dim

Estrogen Metabolites

		Reference Range
2-Hydroxyestrone (24hr urine)	55.5	9.2-76.6 mcg/g Creat.
16α-Hydroxyestrone (24hr urine)	17.7	2.4-20.3 mcg/g Creat.
4-Hydroxyestrone (24hr urine)	23.7	<= 5.3 mcg/g Creat.
2-Methoxyestrone (24hr urine)	2.4	>= 1.7 mcg/g Creat.
4-Methoxyestrone (24hr urine)	<dl	>= 1.9 mcg/g Creat.

Ratios

Anabolic/Catabolic Balance (24hr urine)	0.5	1.0-3.9
11β-HSD Index (24hr urine)	0.36	0.59-1.42
E/A: 5β/5α Ratio (24hr urine)	0.5	0.8-2.6
2-Hydroxyestrone/16α-Hydroxyestrone Ratio	3.1	1.7-2.8
2-Methoxyestrone/2-Hydroxyestrone Ratio	0.04	>= 0.09

What Would You Do Now?

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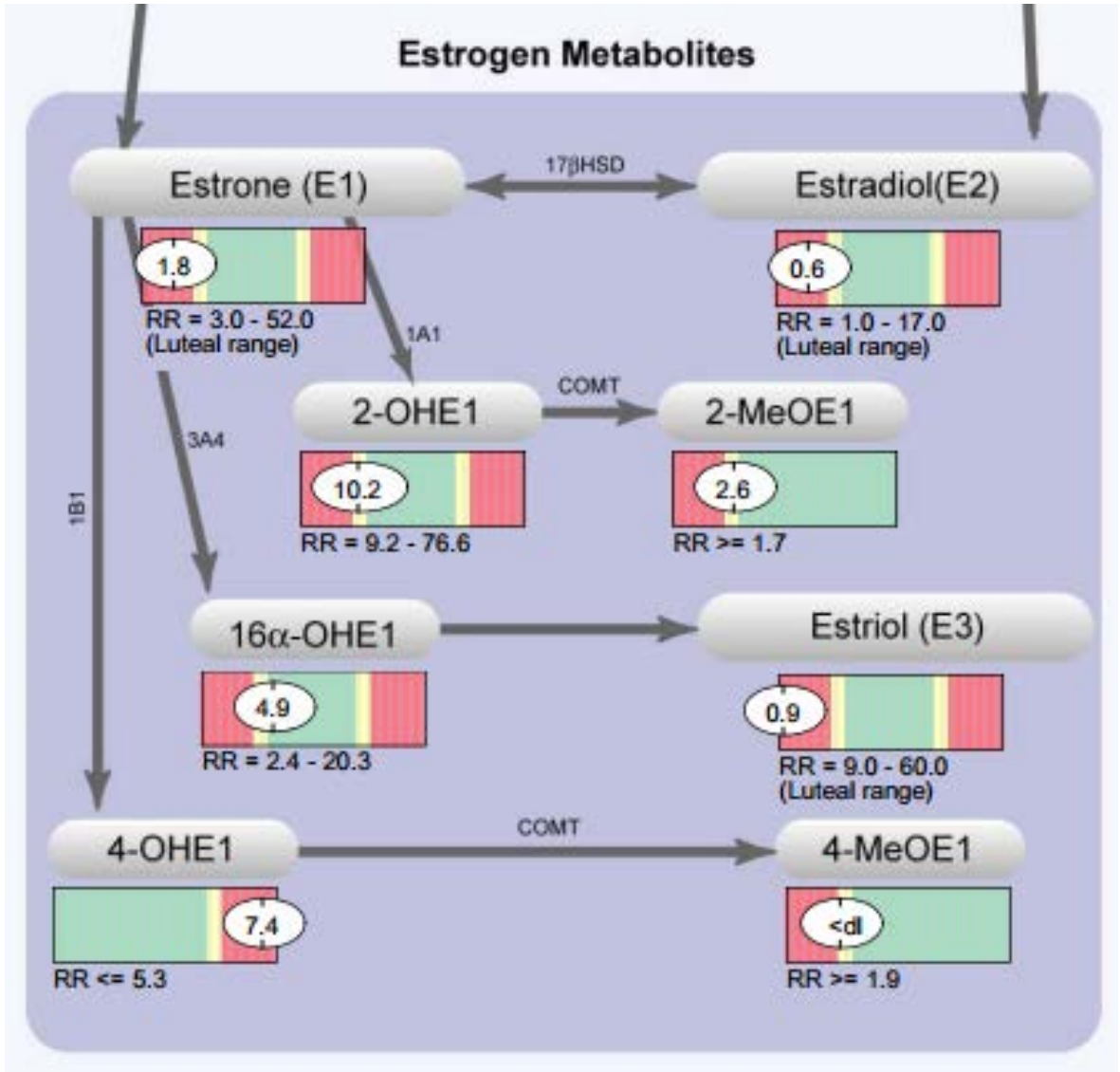
Case #2

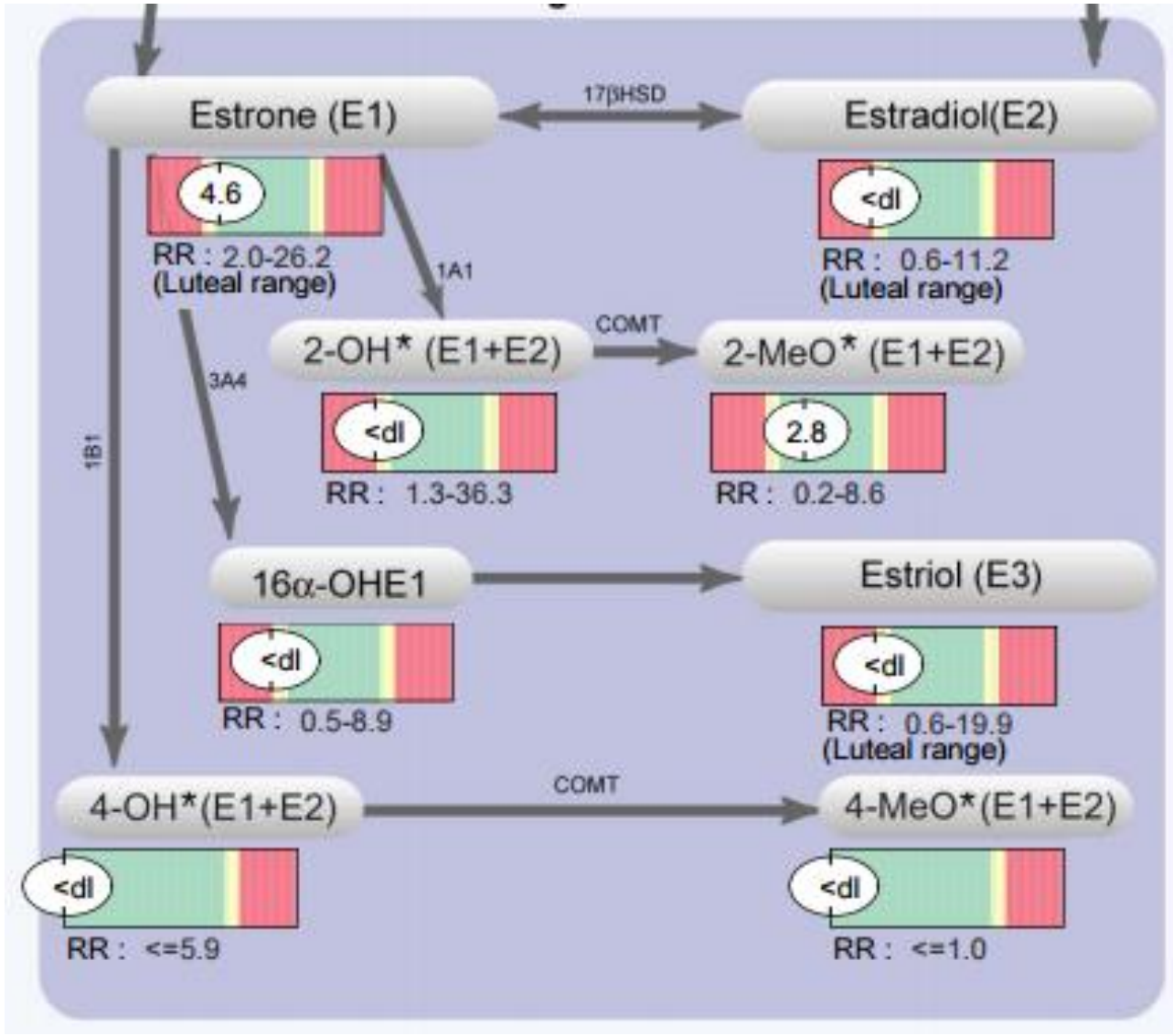
- Pt: 55yo post-menopausal
- History of breast cancer
- Current S/Sx
- Fairly low energy which has improved over last few months
- Some GI complaints, mostly constipation but occasional diarrhea
- Low thyroid function
- High stress levels
- Meds/Supplements


Estrogen Metabolites
Estrone (E1)
17βHSD
Estradiol(E2)
1.8
RR = 3.0 - 52.0
(Luteal range)
0.6
RR = 1.0 - 17.0
(Luteal range)
1A1
COMT
2-OHE1
2-MeOE1
3A4
10.2
RR = 9.2 - 76.6
2.6
RR >= 1.7
1B1
16α-OHE1
Estriol (E3)
4.9
RR = 2.4 - 20.3
0.9
RR = 9.0 - 60.0
(Luteal range)
COMT
4-OHE1
4-MeOE1
7.4
RR <= 5.3
<dl
RR >= 1.9

Case #2 Continued

- Since first test: been doing some methylation support and estrogen detox support
  - 5-MTHF
  - P-5-P
  - Methylcobalamin
  - TMG
  - Turmeric
  - Rosemary
  - Broccoli extract, plus some DIM
- Not on any Rx medications
- Follow-up: Labs much improved
  - Improvement in energy, but is a slow process
  - Continuing to work on stress reduction

Follow-Up Test – 8.16.16



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Thank You
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Michael Chapman, ND
Moderator

Filomena Trindade, MD, MPH
Presenter

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- Diagnostic profiles featured in this webinar
- How Genova's profiles might support patients in your clinical practice
- Review a profile that has already been completed on one of your patients

We look forward to hearing from you!

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Estrogen Metabolism:
Are We Assessing It Properly?

Filomena Trindade, MD, MPH