Breast Cancer Insights:

What Markers to Watch Using the DUTCH Test

Carrie Jones, ND, FABNE, MPH

This lecture and the cited scientific literature, when referring to females/women, are referring to individuals born biological females; when referring to males/men, this lecture is referring to individuals born biological males.

According to Breastcancer.org

- **1 in 8 women** will develop invasive breast cancer in her lifetime

https://www.breastcancer.org/symptoms/understand_bc/statistics

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- In women under 45, breast cancer is more **common in Black women** than white women. Overall, Black women are **more likely to die** of breast cancer dt an aggressive form
- **5-10%** are associated with *known* genetic mutations like **BRCA**
- **85%** of women with breast cancer have **no family history!**

https://www.breastcancer.org/symptoms/understand_bc/statistics

Breast Cancer

Breast cancer is an **unfortunate combination** of genetics, hormones, lifestyle, and environmental factors.

The Objective of the Webinar

Understand key markers in the DUTCH test and how they related to breast cancer in order to possibly mitigate risk.

Key DUTCH Markers and Breast Cancer

1. Estrogen excess
2. Phase 1 metabolites: 4-OH-E1 and 16-OH-E1
3. Phase 2 metabolite: methylation
4. Cortisol Pattern
5. Melatonin
6. Pyroglutamate
7. 8OHdG

Key DUTCH Markers and Breast Cancer

- Please remember, if one or some of these markers are concerning, it does not always immediately indicate cancer. **There is no absolute.**
- Take these results into consideration with the individual in front of you.

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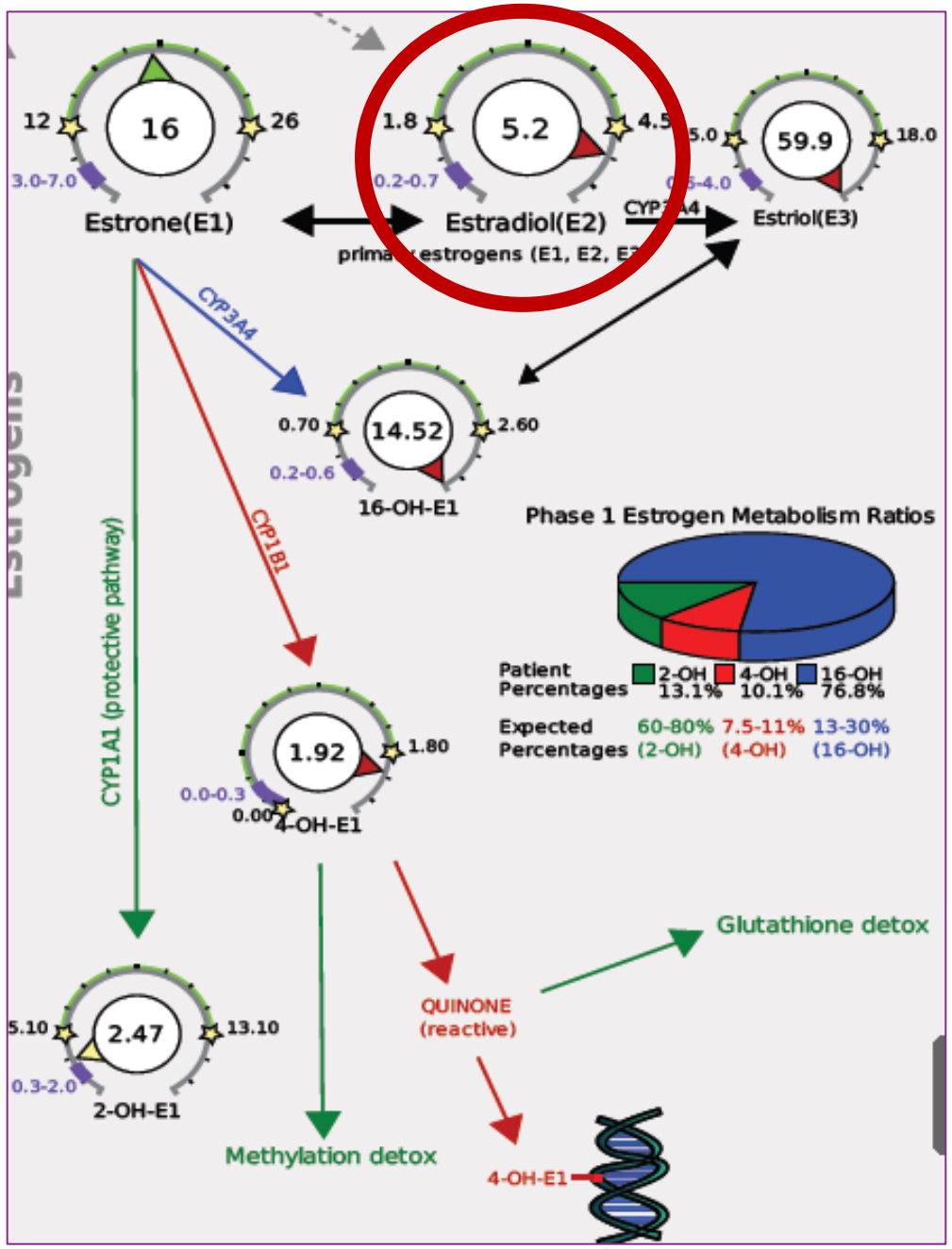

Does estrogen cause cancer? Should someone take estrogen?

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ABOUT US ▾ DUTCH TESTING ▾ RESEARCH PROVIDERS ▾ PATIENTS ▾ EDUCATION ▾ DUTCH NEWS ▾

Dense Breasts and Estrogen

- **Dense breast** = high amounts of glandular and fibrous tissue, lower amounts of fat tissue
- Dense breast tissue as seen on mammogram have an **increased risk of breast cancer**
  - >75% density have 4-6x the risk of cancer
- In a 2018 study, estrogen (ERT) use was **not associated** with dense breasts/breast cancer but **estrogen + progestin was associated**

- Azam S, Lange T, Huynh S, et al. Hormone replacement therapy, mammographic density, and breast cancer risk: a cohort study Cancer Causes Control. 2018; 29(6):495-505.

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Reminder: breast PAIN and DENSITY are different. Estrogen can induce mastalgia

- Azam S, Lange T, Huynh S, et al. Hormone replacement therapy, mammographic density, and breast cancer risk: a cohort study Cancer Causes Control. 2018; 29(6):495-505.

Androgen Excess and Estrogen?

- "Elevated circulating androgen levels are **consistently associated with increased breast cancer risk**, but the underlying mechanism of action is unclear."
- "In breast tumors, AR expression has been detected in up to 85% of cases, although this varies by breast cancer subtype; while almost all ER+ cancers express AR, only 10–35% of triple negative (ER-/PR-/HER2-) breast cancers express AR."

Trabert B, Sherman ME, Kannan N, Stanczyk FZ. Progesterone and Breast Cancer . 2020; 41(2):320-344.

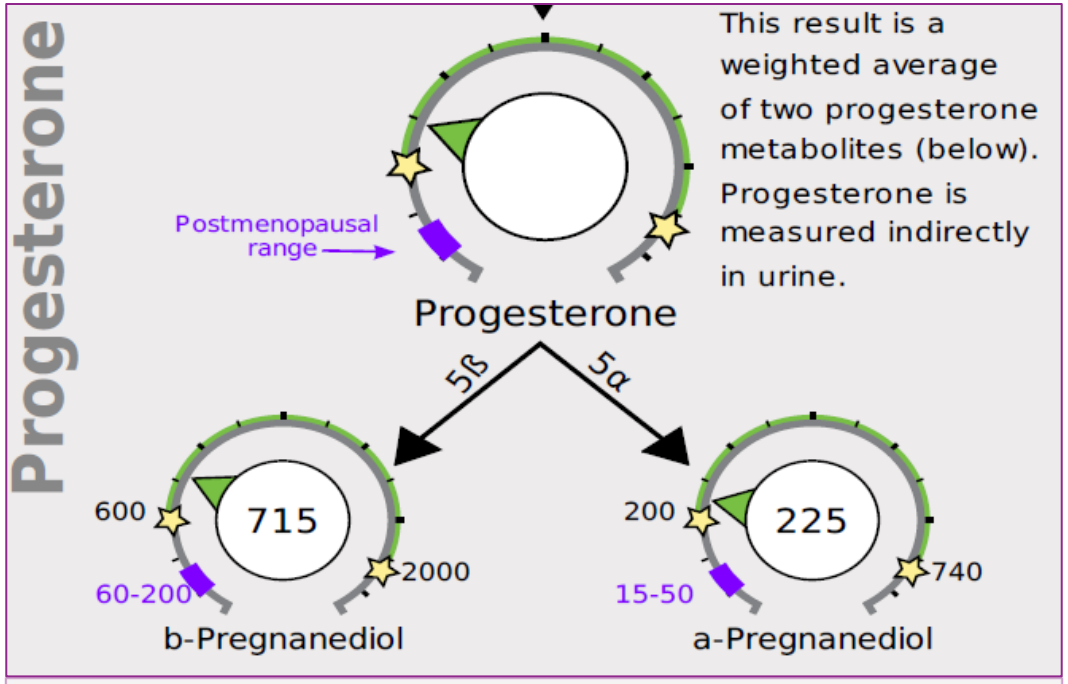
- "Elevated levels of circulating postmenopausal androgens (androstenedione and testosterone) and androgen precursors (dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS)) increase breast cancer risk, with women in the top quartile of these hormones at 2–3 fold increased risk versus women in the lowest quartile. Because these androgens are the obligate precursors for estrogens, it is not known whether their observed effect is independent of the estrogen association. In addition, epidemiologic studies have not examined the functional role of androgen metabolites synthesized within the breast. Indeed, the normal breast contains the steroidogenic enzymes needed to convert circulating precursors to biologically active forms, with the derived 5α-reduced androgens, particularly dihydrotestosterone (DHT), being the most potent. In women, however, levels of circulating DHT are often below assay detection, and do not reflect peripheral 5α-reductase activity. Rather, androsterone glucuronide (ADT-G), a distal metabolite of DHT, together with androstanediol glucuronide (found as 2 isomers: 5α-androstane-3α,17β diol-3-glucuronide (3α-diol-3G) and 5α-androstane-3α,17β diol-17-glucuronide (3α-diol-17G)), **have been shown to reflect <u>total tissue-level androgenic activity</u> better** than the proandrogens (eg, testosterone, androstenedione, etc.)."

Trabert B, Sherman ME, Kannan N, Stanczyk FZ. Progesterone and Breast Cancer . 2020; 41(2):320-344.

Yes. We test that.

Androgens and Metabolites (Urine)				
DHEA-S	Within range	179.0	ng/mg	20 - 750
Androsterone	Above range	2491.0	ng/mg	200 - 1650
Etiocholanolone	High end of range	999.0	ng/mg	200 - 1000
Testosterone	Above range	17.3	ng/mg	2.3 - 14
5a-DHT	Above range	19.8	ng/mg	0 - 6.6
5a-Androstanediol	High end of range	29.8	ng/mg	12 - 30
5b-Androstanediol	Within range	55.0	ng/mg	20 - 75
Epi-Testosterone	Above range	19.1	ng/mg	2.3 - 14

What about Progesterone?



Progesterone: It's Complicated

Endocr Rev. 2020 Apr; 41(2): 320–344.
Published online 2019 May 2. doi: 10.1210/endrev/bnz001

PMCID: PMC7156851
PMID: 31512725

Progesterone and Breast Cancer

Britton Trabert,[1] Mark E Sherman,[2] Nagarajan Kannan,[3] and Frank Z Stanczyk[4]

Abstract

Go to:

Synthetic progestogens (progestins) have been linked to increased breast cancer risk; however, the role of endogenous progesterone in breast physiology and carcinogenesis is less clearly defined. Mechanistic studies using cell culture, tissue culture, and preclinical models implicate progesterone in breast carcinogenesis. In contrast, limited epidemiologic data generally do not show an association of circulating progesterone levels with risk, and it is unclear whether this reflects methodologic limitations or a truly null relationship. Challenges related to defining the role of progesterone in breast physiology and neoplasia include: complex interactions with estrogens and other hormones (eg, androgens, prolactin, etc.), accounting for timing of blood collections for hormone measurements among cycling women, and limitations of assays to measure progesterone metabolites in blood and progesterone receptor isotypes (PRs) in tissues. Separating the individual effects of estrogens and progesterone is further complicated by the partial dependence of *PR* transcription on estrogen receptor (ER)α-mediated transcriptional events; indeed, interpreting the integrated interaction of the hormones may be more essential than isolating independent effects. Further, many of the actions of both estrogens and progesterone, particularly in "normal" breast tissues, are driven by paracrine mechanisms in which ligand binding to receptor-positive cells evokes secretion of factors that influence cell division of neighboring receptor-negative cells. Accordingly, blood and tissue levels may differ, and the latter are challenging to measure. Given conflicting data related to the potential role of progesterone in breast cancer etiology and interest in blocking progesterone action to prevent or treat breast cancer, we provide a review of the evidence that links progesterone to breast cancer risk and suggest future directions for filling current gaps in our knowledge.

Let’s Focus on Estrogen Metabolism

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Clin Transl Med. 2016; 5: 12.
Published online 2016 Mar 15. doi: 10.1186/s40169-016-0088-3

PMCID: PMC4792821
PMID: 26979321

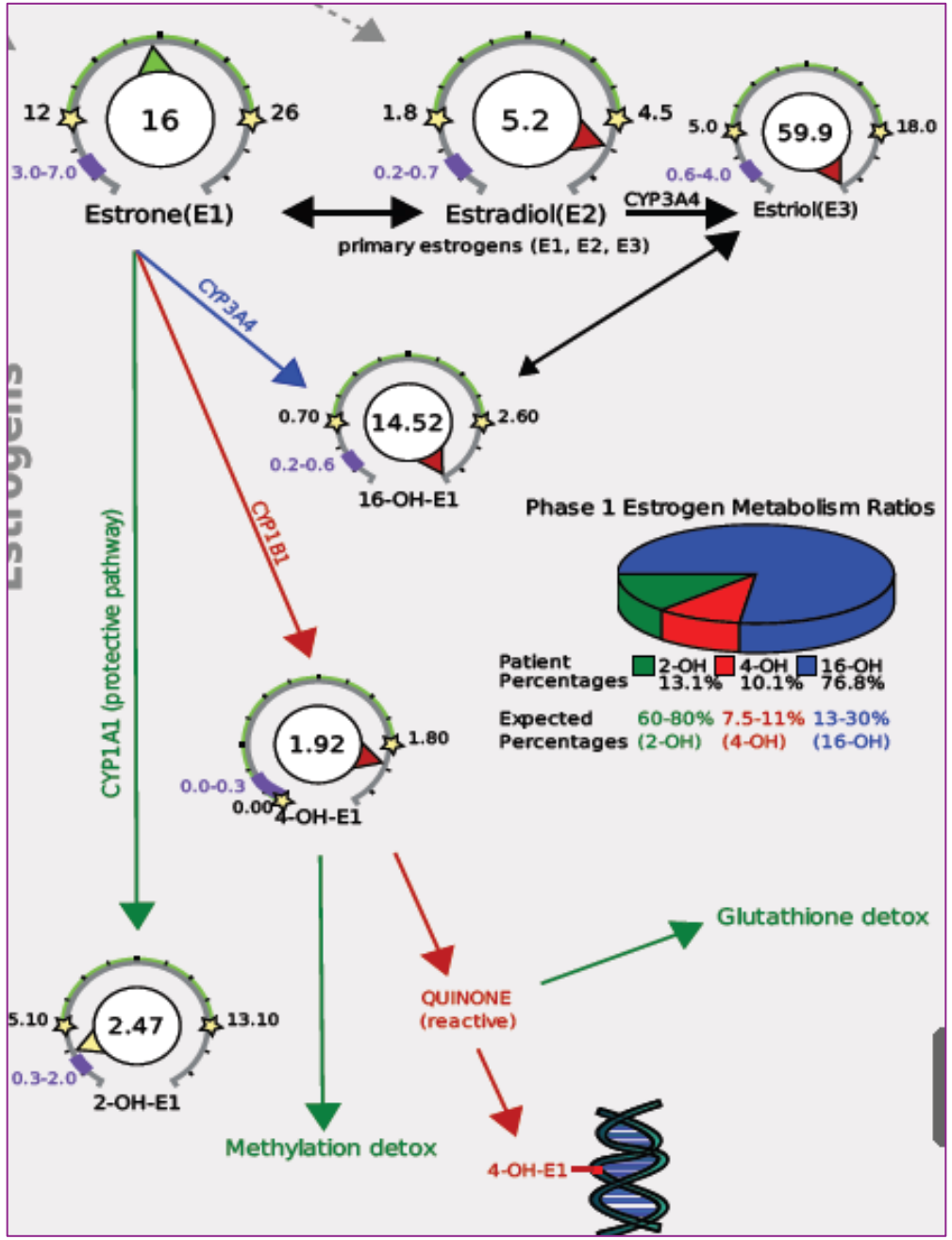
Depurinating estrogen-DNA adducts, generators of cancer initiation: their minimization leads to cancer prevention

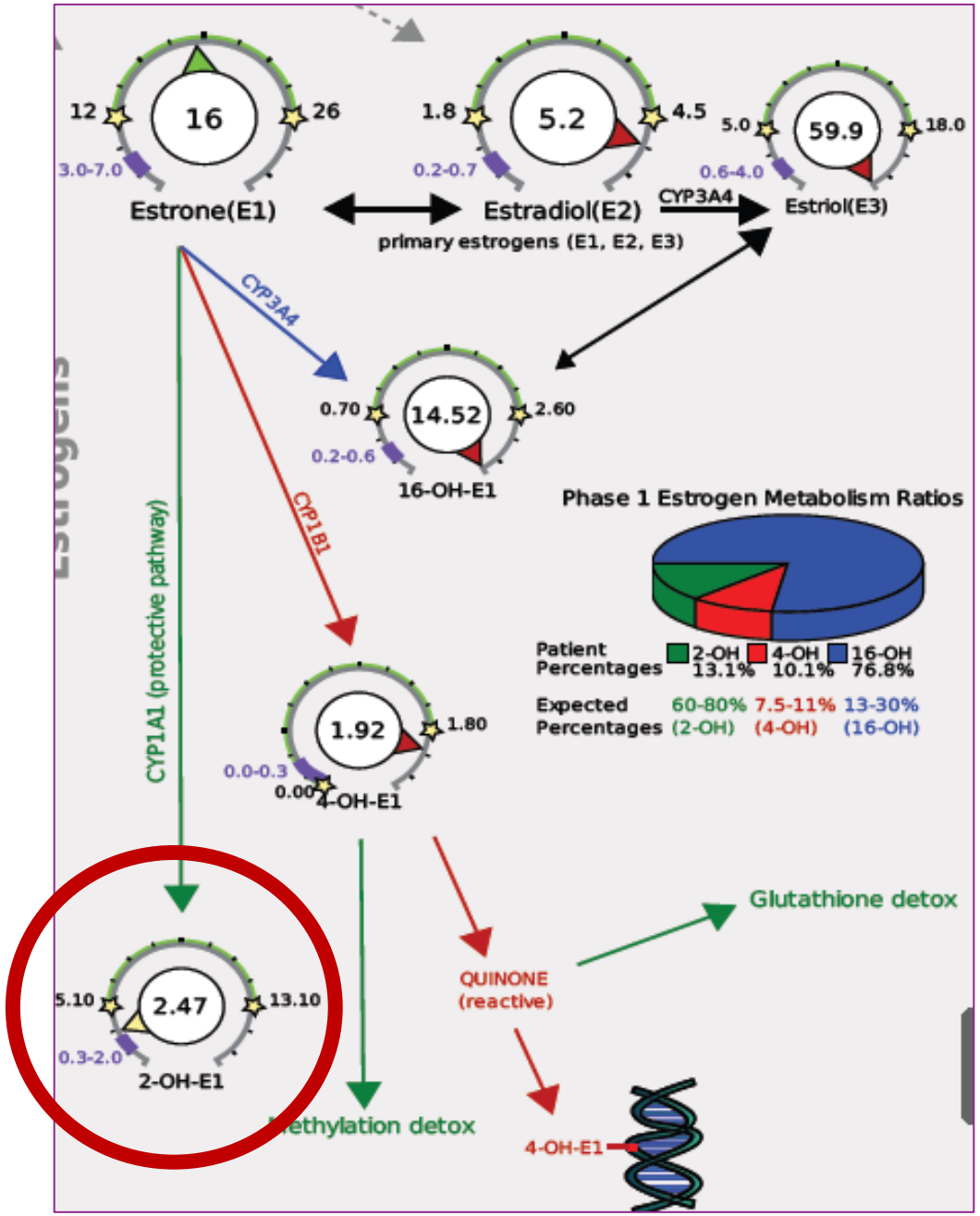
Ercole L. Cavalieri and Eleanor G. Rogan

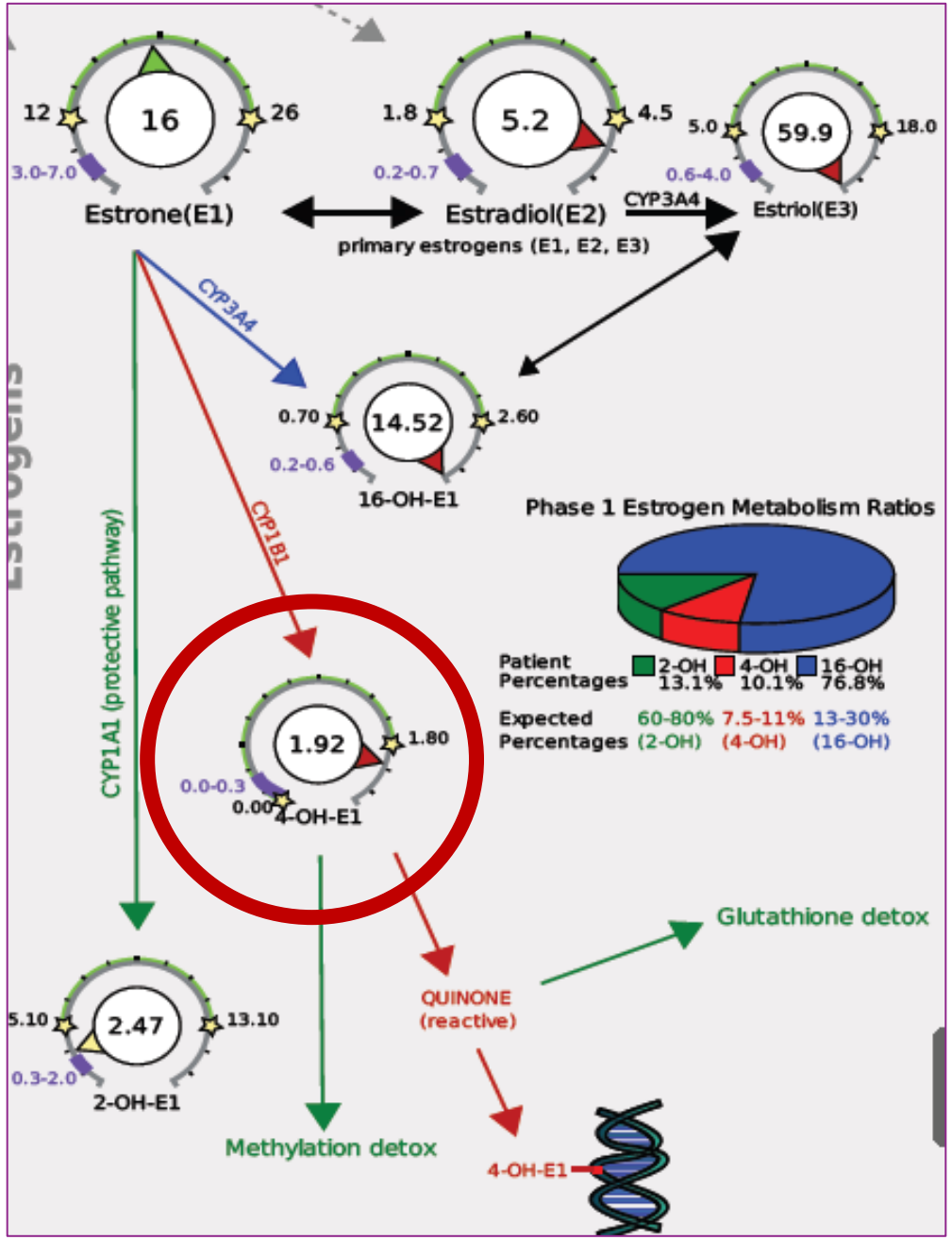
Abstract

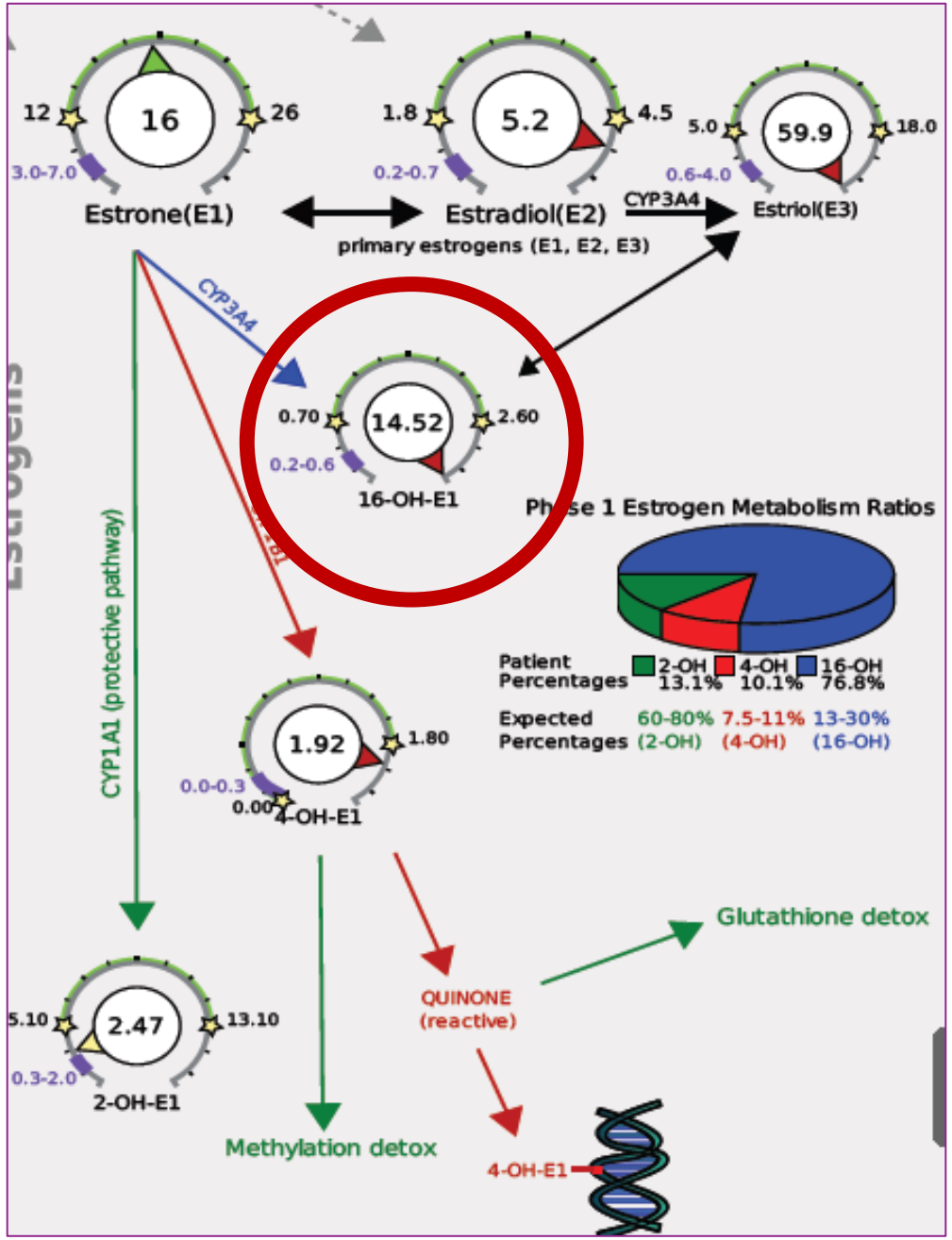
Go to:

Estrogens can initiate cancer by reacting with DNA. Specific metabolites of endogenous estrogens, the 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. A variety of endogenous and exogenous factors can disrupt estrogen homeostasis, which is the normal balance between estrogen activating and protective enzymes. In fact, if estrogen metabolism becomes unbalanced and generates excessive catechol estrogen 3,4-quinones, formation of depurinating estrogen-DNA adducts increases and the risk of initiating cancer is greater. The levels of depurinating estrogen-DNA adducts are high in women diagnosed with breast cancer and those at high risk for the disease. High levels of depurinating estrogen-DNA adducts before the presence of breast cancer indicates that adduct formation is a critical factor in breast cancer initiation. Women with thyroid or ovarian cancer also have high levels of estrogen-DNA adducts, as do men with prostate cancer or non-Hodgkin lymphoma. Depurinating estrogen-DNA adducts are initiators of many prevalent types of human cancer. These findings and other discoveries led to the recognition that reducing the levels of estrogen-DNA adducts could prevent the initiation of human cancer. The dietary supplements *N*-acetylcysteine and resveratrol inhibit formation of estrogen-DNA adducts in cultured human breast cells and in women. These results suggest that the two supplements offer an approach to reducing the risk of developing various prevalent types of human cancer.









Estrogen Metabolite: 2-OHE1

- CYP1A activity primarily in the liver, but also extra-hepatically
- Considered the ***"less carcinogenic"*** toxic metabolite
  - Binds to ERs with ***less affinity, not as tightly***
  - Forms ***'stable' adducts*** with DNA – less likely to result in mutations

Estrogen Metabolite: 4-OHE1

- CYP1B1 activity primarily in the breast, ovary, uterus, lung and kidney, minorly in the liver
- Binds to the estrogen receptor with **higher and tighter affinity** than 2-OHE1 but not as much as 16-OHE1
- Are considered **free radicals** in their semi-quinone or quinone states
- **Considered *much more carcinogenic*** because they form ‘unstable’ or ‘depurinating’ adducts – resulting in increased risk of mutation
  - However, this is mixed in some literature

Estrogen Metabolite: 16-OHE1

- Metabolite of Estrone (via CYP3A4) that can also become Estriol
- Binds ***tightly/high affinity*** for the estrogen receptor
- It is thought to be **proliferative** and can stimulate cell proliferation in someone with breast cancer

Citations:

- Sisti JS, Hankinson SE, Caporaso NE, et al. Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2015; 24(8):1174-83. [pubmed]
- Sampson JN, Falk RT, Schairer C, et al. Association of Estrogen Metabolism with Breast Cancer Risk in Different Cohorts of Postmenopausal Women Cancer Res. 2017; 77(4):918-925.
- Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human CYP1B1 is regulated by estradiol via estrogen receptor. Cancer research. 2004; 64(9):3119-25. [pubmed]
- Cavalieri E, Rogan E. The 3,4-Quinones of Estrone and Estradiol Are the Initiators of Cancer whereas Resveratrol and N-acetylcysteine Are the Preventers IJMS. 2021; 22(15):8238-.
- Eliassen AH, Missmer SA, Tworoger SS, Hankinson SE. Circulating 2-Hydroxy- and 16α-Hydroxy Estrone Levels and Risk of Breast Cancer among Postmenopausal Women Cancer Epidemiol Biomarkers Prev. 2008; 17(8):2029-2035.

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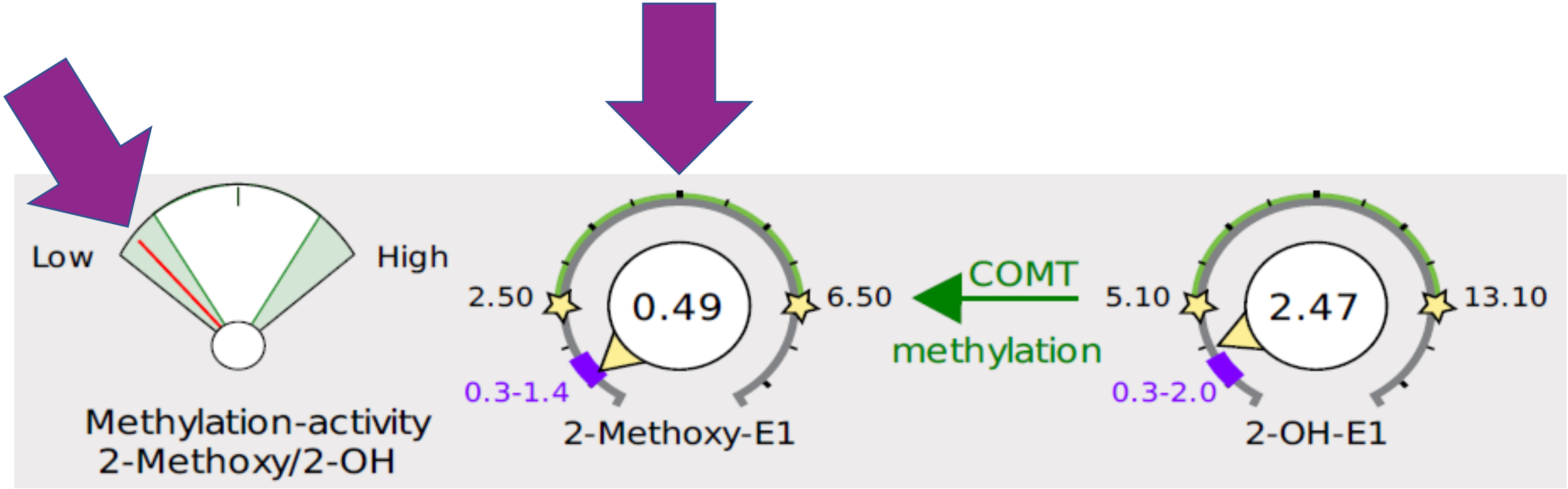

Estrogen Metabolite: 2-MethoxyE1

- 2-OHE1 is methylated via **COMT** (using SAM and Mg)
  - Low methylation can lead to phase 1 metabolites heading down the Quinone/adduct pathway.
- Considered ***anti-proliferative*** through microtuble disruption, apoptosis induction and angiogenesis inhibition
- Considered to have ***anti-aromatase*** activity
- Not thought to bind to the estrogen receptor (much)

Citations:

- Lakhani NJ, Sarkar MA, Venitz J, Figg WD. 2-Methoxyestradiol, a promising anticancer agent. Pharmacotherapy. 2003; 23(2):165-72. [pubmed]
- Purohit A, Singh A, Ghilchik MW, Reed MJ. Inhibition of tumor necrosis factor alpha-stimulated aromatase activity by microtubule-stabilizing agents, paclitaxel and 2-methoxyestradiol. Biochemical and biophysical research communications. 1999; 261(1):214-7. [pubmed]

Working on Estrogen Metabolism

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Estrogen Metabolism Start to Finish

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Webinar Event
July 15, 2020
12 p.m. PST / 3 p.m. EST

Estrogen Metabolism: Start to Finish

Presented by Tara Scott, MD, FACOG, FAAFM, ABOIM, CNMP

Unhealthy estrogen metabolism is often to blame for estrogen related diseases and conditions. The breakdown and excretion of estrogens is an extremely complicated process with a wide range of influencing factors.

This webinar will:

- Discuss the phases and epigenetic effects of estrogen metabolism
- Examines suboptimal estrogen detoxification ways to improve it
- Review specific case examples

Watch on YouTube

Estrogen Metabolism Highlights

- Learn it as phase 1 → 2 → 3
- **Address it as 3 → 2 → 1**
- **Phase 3** can be evaluated in **stool testing**
- **Phase 2** involves **COMT**, it's co-factor SAM and co-nutrient, magnesium
  - It also involves several other enzymes/snps and nutrients on the methionine cycle
- **Phase 1** involves several CYP p450 enzymes
  - It is shifted through supplements such as I3C, DIM and Quercetin
- Supporting **Quinone Reductase (NQ01)** and **Glutathione-S-Transferase** helps too

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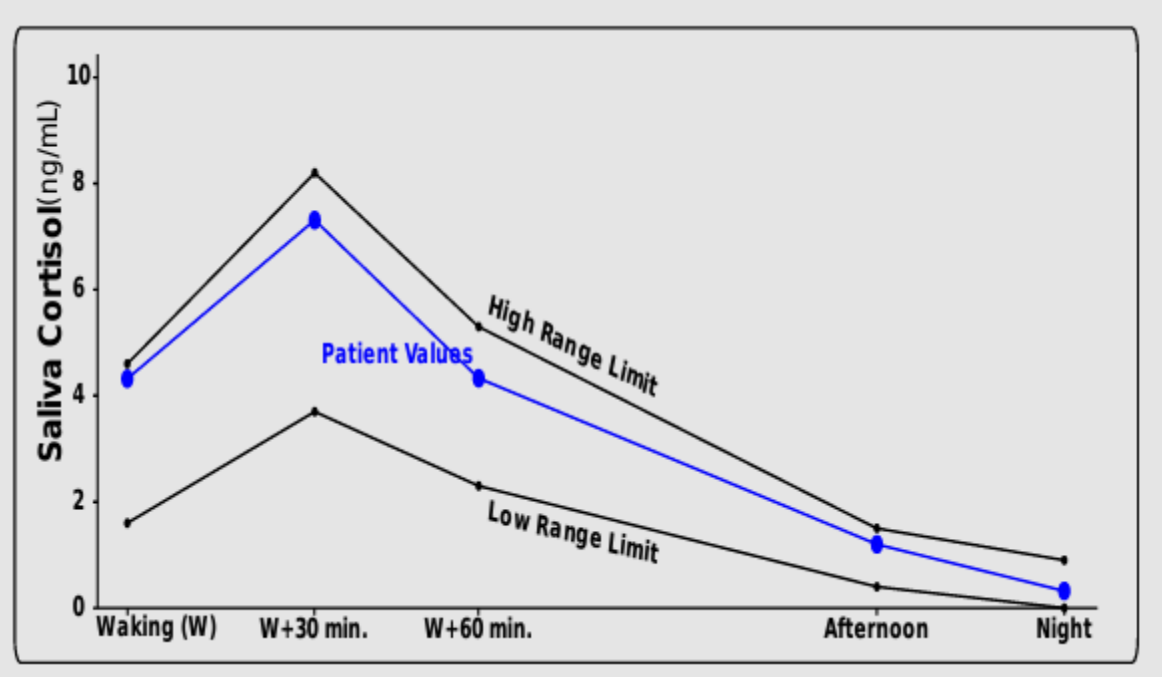
Circadian Rhythm

- A normal circadian rhythm starts with a cortisol awakening response then falls throughout the day.



Cash et al. Journal of Experimental & Clinical Cancer Research (2021) 40:119
https://doi.org/10.1186/s13046-021-01919-5

Journal of Experimental & Clinical Cancer Research

REVIEW Open Access

The role of the circadian clock in cancer hallmark acquisition and immune-based cancer therapeutics

Elizabeth Cash[1*], Sandra Sephton[2], Cassandra Woolley[3], Attia M. Elbehi[4], Anu R. I.[5], Bene Ekine-Afolabi[6,7] and Victor C. Kok[8,9]

Abstract

The circadian system temporally regulates physiology to maintain homeostasis. Co-opting and disrupting circadian signals appear to be distinct attributes that are functionally important for the development of a tumor and can enable or give rise to the hallmarks that tumors use to facilitate their initiation, growth and progression. Because circadian signals are also strong regulators of immune cell proliferation, trafficking and exhaustion states, they play a role in how tumors respond to immune-based cancer therapeutics. While immuno-oncology has heralded a paradigm shift in cancer therapeutics, greater accuracy is needed to increase our capability of predicting who will respond favorably to, or who is likely to experience the troubling adverse effects of, immunotherapy. Insights into circadian signals may further refine our understanding of biological determinants of response and help answer the fundamental question of whether certain perturbations in circadian signals interfere with the activity of immune checkpoint inhibitors. Here we review the body of literature highlighting circadian disruption as a cancer promoter and synthesize the burgeoning evidence suggesting circadian signals play a role in how tumors respond to immune-based anti-cancer therapeutics. The goal is to develop a framework to advance our understanding of the relationships between circadian markers, cancer biology, and immunotherapeutics. Bolstered by this new understanding, these relationships may then be pursued in future clinical studies to improve our ability to predict which patients will respond favorably to, and avoid the adverse effects of, traditional and immune-based cancer therapeutics.

Keywords: Circadian, Cancer, Immuno-oncology, Immune checkpoint inhibitor, Glucocorticoid, Clock gene



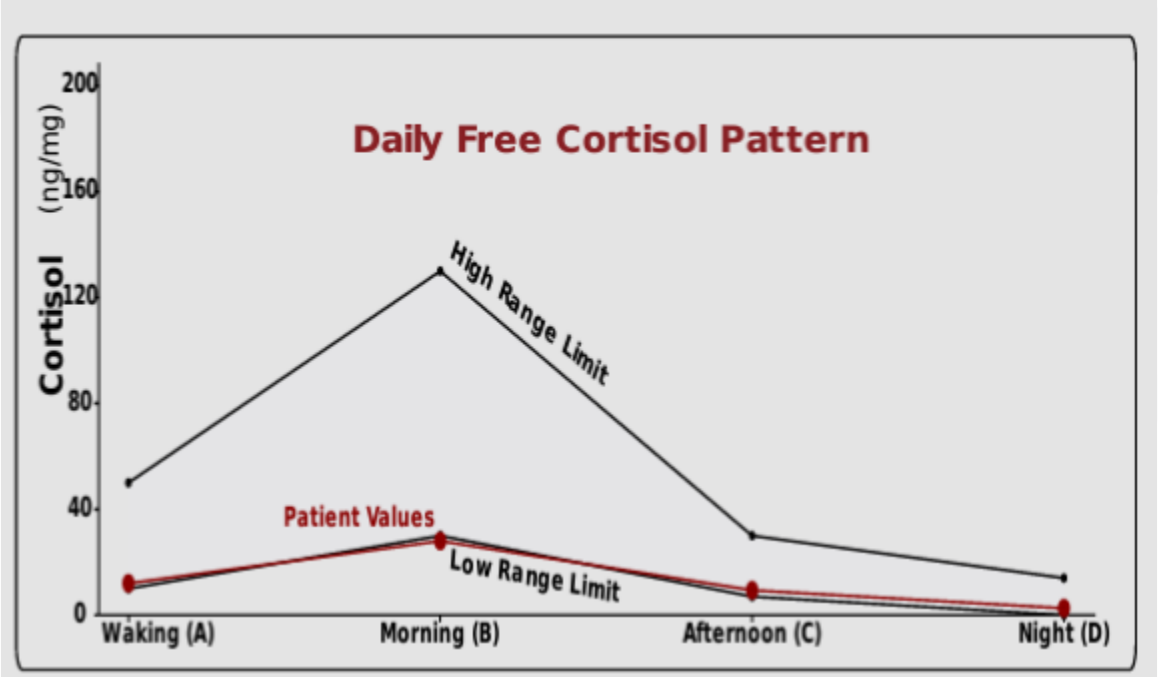

Shift workers

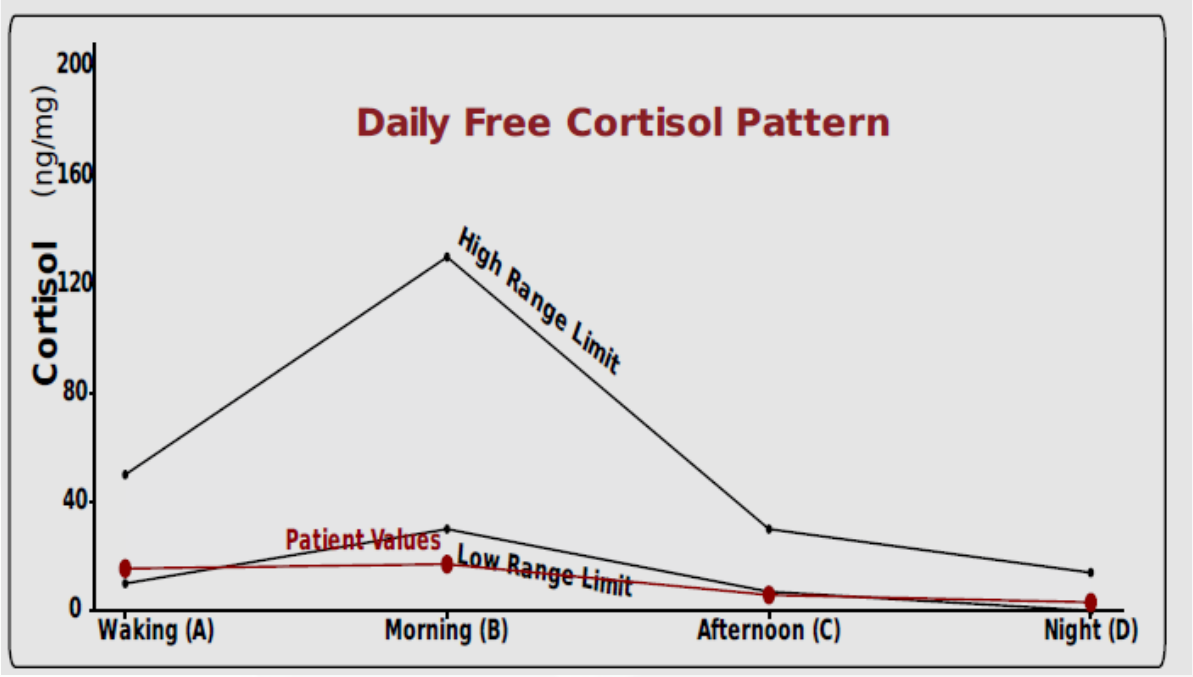
Shift Workers and Circadian Rhythm?

- Shift workers are crucial for making the world go round. We appreciate our shift workers. But…
- The research leans towards them having a **higher risk for breast cancer development** likely due to the circadian rhythm dysfunction
  - The International Agency for Research on Cancer has listed shift work as a **possible carcinogen.**

- Jones ME, Schoemaker MJ, McFadden EC, Wright LB, Johns LE, Swerdlow AJ. Night shift work and risk of breast cancer in women: the Generations Study cohort Br J Cancer. 2019; 121(2):172-179.
- Manouchehri, E., Taghipour, A., Ghavami, V. *et al.* Night-shift work duration and breast cancer risk: an updated systematic review and meta-analysis. *BMC Women's Health* **21,** 89 (2021). https://doi.org/10.1186/s12905-021-01233-4
- Szkiela M, Kusideł E, Makowiec-Dąbrowska T, Kaleta D. Night Shift Work—A Risk Factor for Breast Cancer IJERPH. 2020; 17(2):659-.

What about the slope of the cortisol curve?





Clinical Trial > Psychoneuroendocrinology. 2004 Sep;29(8):1082-92.
doi: 10.1016/j.psyneuen.2003.11.003.

Flattened cortisol rhythms in metastatic breast cancer patients

Heather C Abercrombie [1], Janine Giese-Davis, Sandra Sephton, Elissa S Epel, Julie M Turner-Cobb, David Spiegel

Affiliations + expand

PMID: 15219660 DOI: 10.1016/j.psyneuen.2003.11.003

Abstract

Allostatic load, the physiological accumulation of the effects of chronic stressors, has been associated with multiple adverse health outcomes. Flattened diurnal cortisol rhythmicity is one of the prototypes of allostatic load, and has been shown to predict shorter survival among women with metastatic breast cancer. The current study compared diurnal cortisol slope in 17 breast cancer patients and 31 controls, and tested associations with variables previously found to be related to cortisol regulation, i.e, abdominal adiposity, perceived stress, social support, and explicit memory. Women with metastatic breast cancer had significantly flatter diurnal cortisol rhythms than did healthy controls. Patients with greater disease severity showed higher mean cortisol levels, smaller waist circumference, and a tendency toward flatter diurnal cortisol rhythms. There were no relations between cortisol slope and psychological or cognitive functioning among patients. In contrast, controls with flatter rhythms showed the expected allostatic load profile of larger waist circumference, poorer performance on explicit memory tasks, lower perceived social support, and a tendency toward higher perceived stress. These findings suggest that the cortisol diurnal slope may have important but different correlates in healthy women versus those with breast cancer.

Psychoneuroendocrinology. 2020 May;115:104648. doi: 10.1016/j.psyneuen.2020.104648. Epub 2020 Mar 8.

Evening salivary cortisol as a single stress marker in women with metastatic breast cancer

Santiago Allende [1], Johnna L Medina [2], David Spiegel [2], Jamie M Zeitzer [2]

Affiliations + expand

PMID: 32171899 DOI: 10.1016/j.psyneuen.2020.104648

Abstract

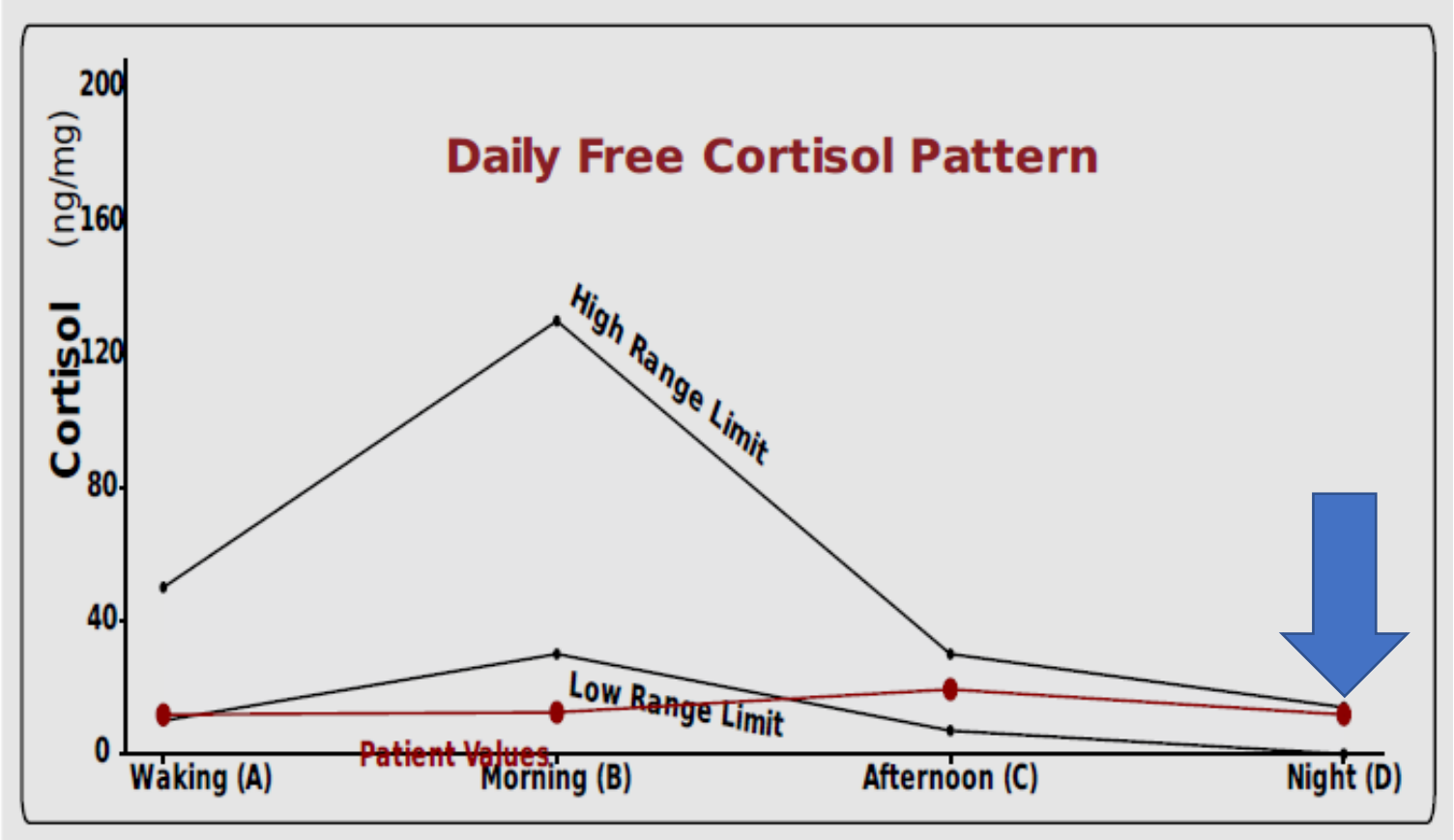
Background: Flattened diurnal salivary cortisol patterns predict shorter subsequent survival with breast, lung, and renal cell carcinomas. The underlying cause of this flattened slope is undetermined, though it has been hypothesized to be secondary to a deficit in the amplitude of the circadian clock. To gain greater insight into the portions of the diurnal salivary curve that are associated with cancer survival, we examined (1) which points in the diurnal curve are predictive of the slope of the curve and (2) whether elevated evening cortisol levels alone are associated with reduced HPA-axis feedback inhibition (i.e., decreased sensitivity to the dexamethasone suppression test).

Method: We examined study hypotheses on adult women with advanced breast cancer (age = 54.3 ± 9.58 years; n = 99) using non-parametric Wilcoxon's rank-sum tests, Spearman correlation coefficients and an accuracy formula based on a confusion matrix. Cortisol was sampled five times per day for three consecutive days, with dexamethasone administered late on the second day.

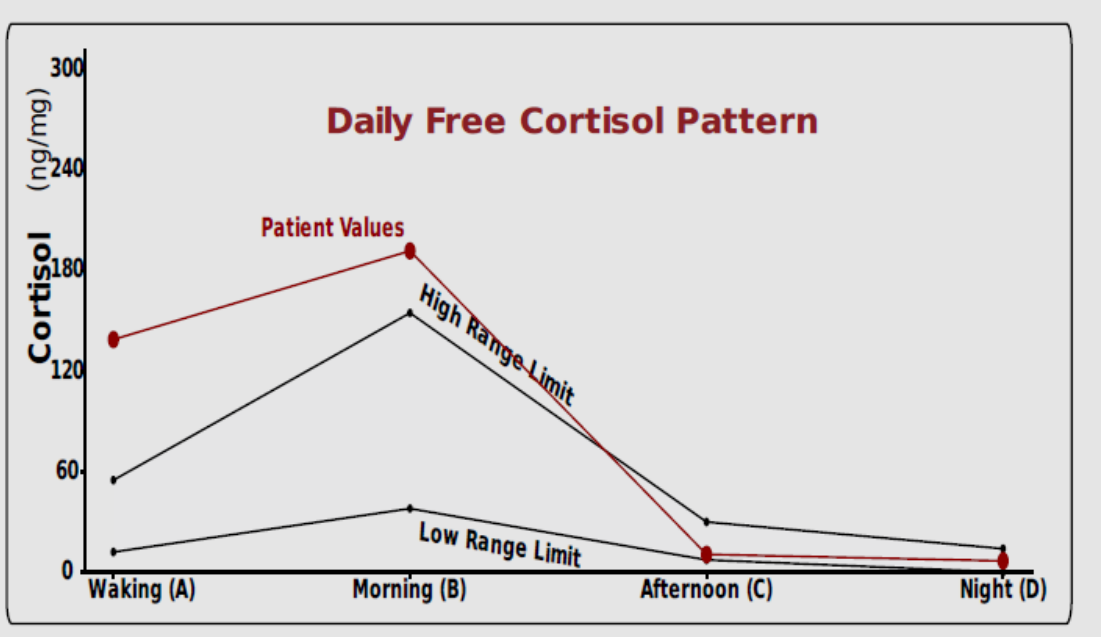
Results: Salivary cortisol concentrations did not vary between those with flat and steep slopes during the morning (p's > .05), but did vary in the evening (p's < 0.05). Furthermore, the concentration of the 2100h alone was 86% accurate in discriminating between individuals classified as having "flat" or "steep" slopes. Dexamethasone suppression was only associated with diurnal salivary cortisol slope (p = .0042).

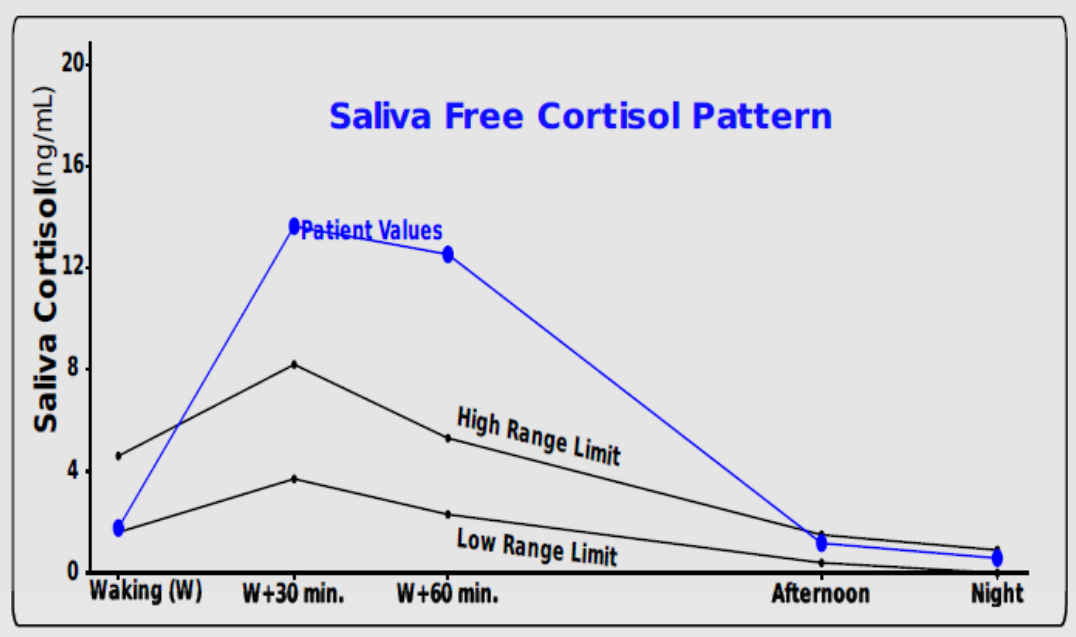
Conclusions: Evening cortisol levels are a sensitive indicator flattened diurnal cortisol slope, suggesting evening cortisol may also be a useful predictor of breast cancer survival. Future research should focus on determining the causes of abnormally increased evening cortisol.

Female in her 50's, night cortisol rising



What about high cortisol?





Elevated Cortisol?

- Some tumor metastases have an increased number of glucocorticoid receptors (GR).
- Cortisol (stress) stimulates these GR.
- This increases colonization and heterogeneity of the cancer cells leading to **shortened survival** of someone with cancer.

Milan M. S. Obradović, Baptiste Hamelin, Nenad Manevski, Joana Pinto Couto, Atul Sethi, Marie-May Coissieux, Simone Münst, Ryoko Okamoto, Hubertus Kohler, Alexander Schmidt, Mohamed Bentires-Alj. Glucocorticoids promote breast cancer metastasis. *Nature*, 2019; DOI: 10.1038/s41586-019-1019-4

Improving the circadian rhythm

- Work on the **stress response** however that looks
- Get **full spectrum light** on waking
- Consider taking **am supplements within 30min** of waking for the CAR
- **Minimize blue/white light** exposure in the evening
- Wear blue light blocking **glasses**
- Sleep in complete **darkness**
- Get exposure to **sunset light**
- **Avoid caffeine/alcohol** before bed
- Evaluate **melatonin** on the DUTCH test
- Consider **calming herbs/nutrients** before bed

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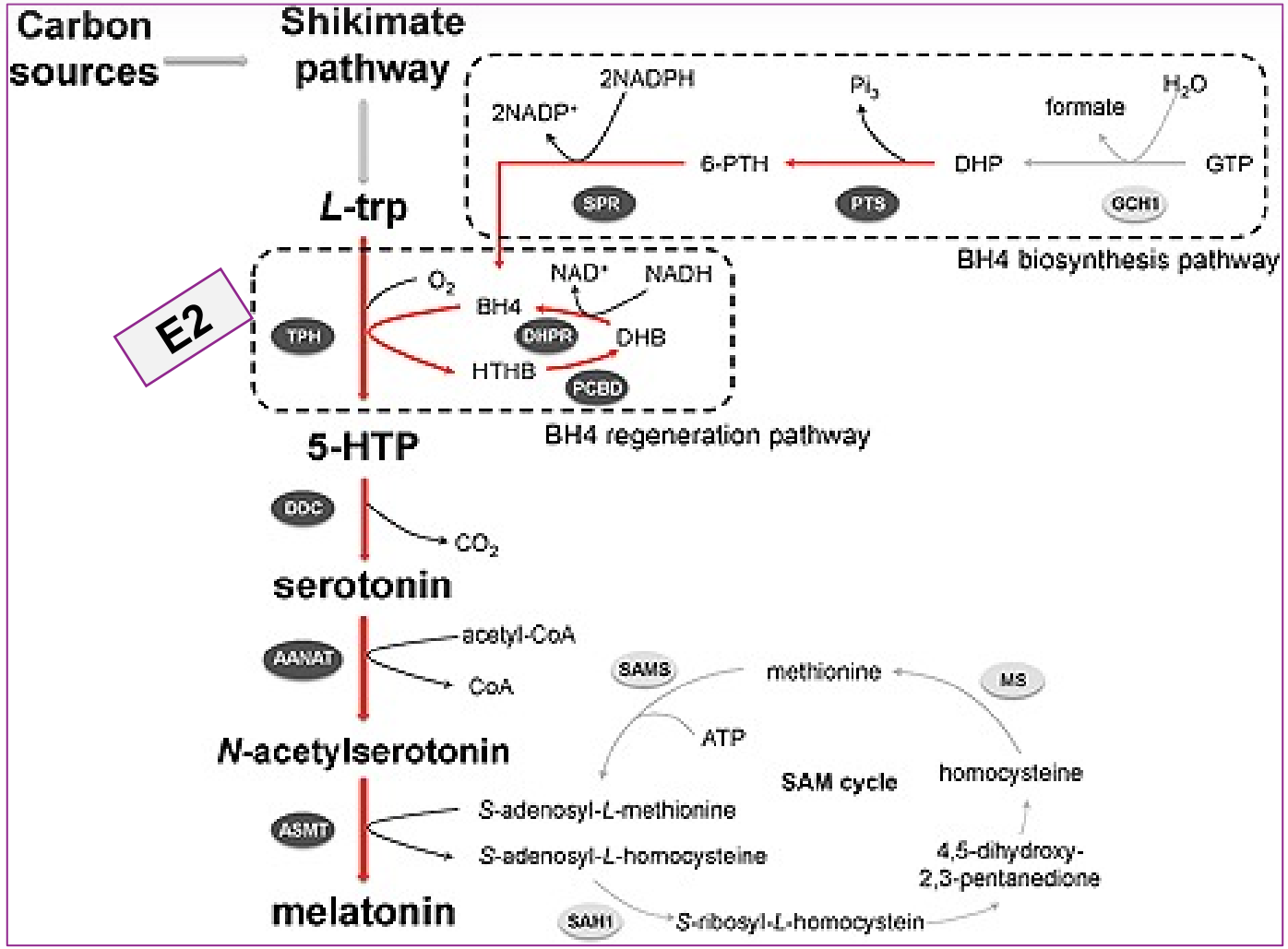
7. 8OHdG

How is Melatonin made?



Germann SM, Baallal Jacobsen SA, Schneider K, et al. Glucose-based microbial production of the hormone melatonin in yeast Saccharomyces cerevisiae. Biotechnol J. 2016; 11(5):717-24. [PDF]

This becomes
6-OH-melatonin-Sulfate (aMT6s),
and is urinated out.

Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
Melatonin* (Waking)	Within range	33.5	ng/mg	10 - 85

Melatonin Facts:

- "Because of its presence in bacteria, which evolved several billion years ago, we have speculated that melatonin is ***phylogenetically the oldest antioxidant in existence.***" *(Reiter et al, 2018)*

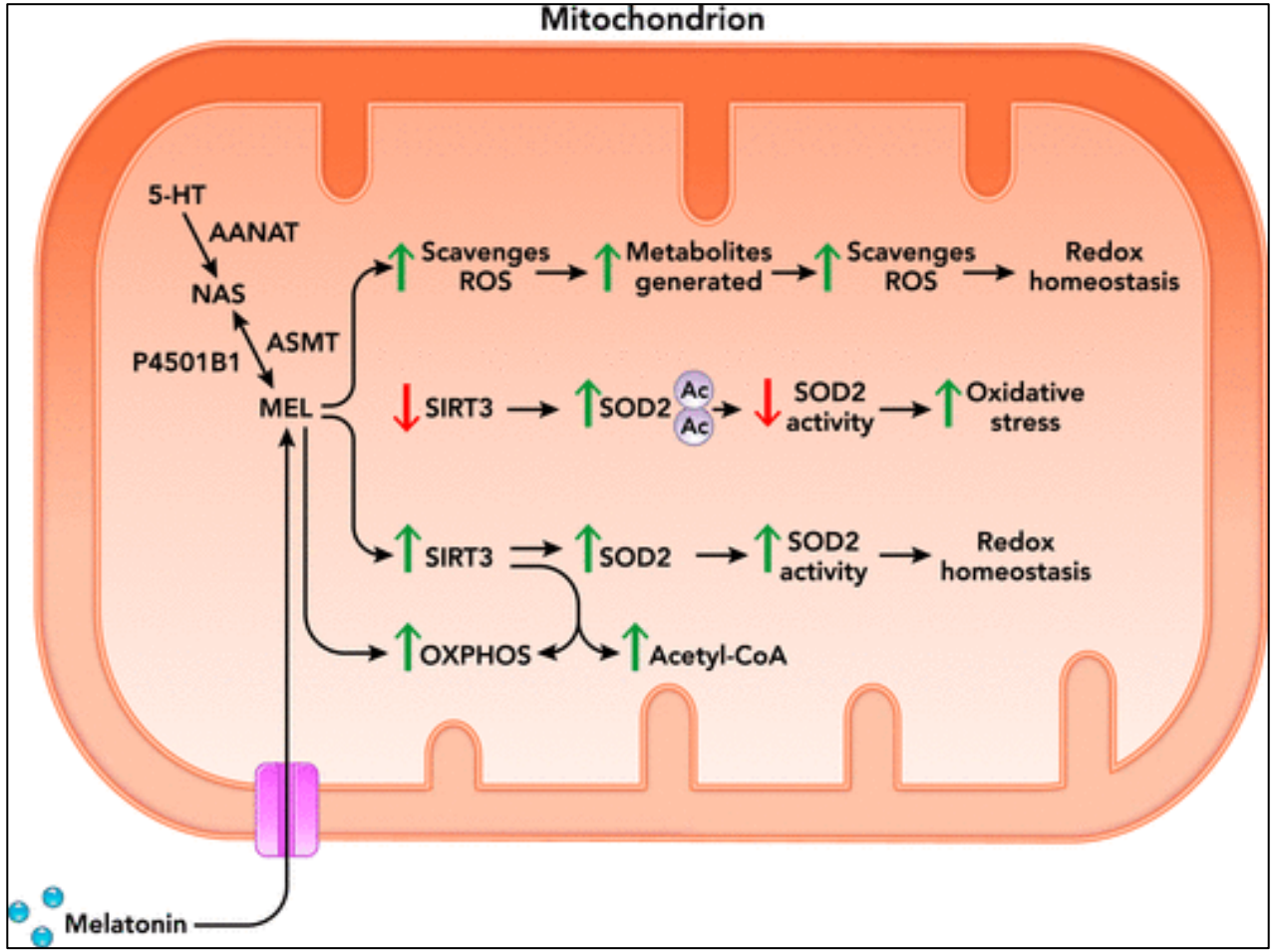
Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XJ, Xu B. Mitochondria: Central Organelles for Melatonin's Antioxidant and Anti-Aging Actions. Molecules. 2018; 23(2):. [PDF]

Melatonin Facts:

- It's a major part of the **circadian rhythm**
  - Circulating levels are primarily made in the gut but also the pineal gland
- Adult tend to make about **0.5mg/day physiologically**
- It is **heavily concentrated** in mitochondria
  - It is now accepted that mitochondria make their own melatonin.
- It can act as a **direct free radical scavenger**

Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XJ, Xu B. Mitochondria: Central Organelles for Melatonin's Antioxidant and Anti-Aging Actions. Molecules. 2018; 23(2):. [PDF]

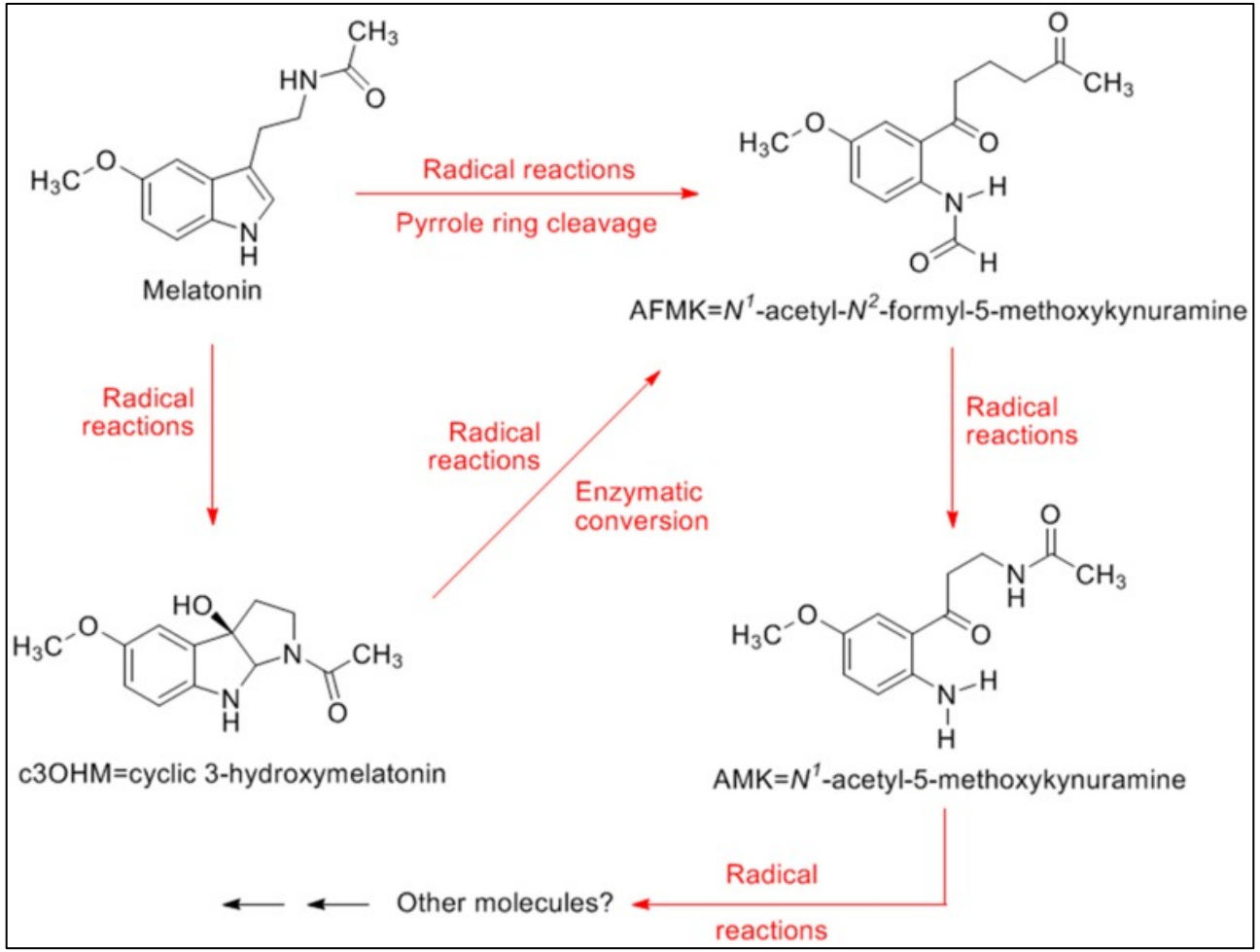
Melatonin saving the day!



Reiter RJ, Ma Q, Sharma R. Melatonin in Mitochondria: Mitigating Clear and Present Dangers Physiology. 2020; 35(2):86-95.

Melatonin and its antioxidant crew

When melatonin meets and disarms a toxin, it gets metabolized into other melatonin metabolites that can also detoxify free radicals. It's the antioxidant cascade.



Reiter RJ, Ma Q, Sharma R. Melatonin in Mitochondria: Mitigating Clear and Present Dangers Physiology. 2020; 35(2):86-95.

Melatonin: an inhibitor of breast cancer

Steven M Hill[1,3,4,5], Victoria P Belancio[1,3,4,5], Robert T Dauchy[1,3,4,5], Shulin Xiang[1,3,4,5], Samantha Brimer[2], Lulu Mao[1,3,4,5], Adam Hauch[2], Peter W Lundberg[2], Whitney Summers[1], Lin Yuan[1,3], Tripp Frasch[1,5] and David E Blask[1,3,4,5]

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[2]Department of Surgery, [3]Tulane Cancer Center and Louisiana Cancer Research Consortium, [4]Circadian Cancer Biology Group, and [5]Tulane Center for Circadian Biology, Tulane University School of Medicine, New Orleans, Louisiana 70112, USA

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Abstract

The present review discusses recent work on melatonin-mediated circadian regulation, the metabolic and molecular signaling mechanisms that are involved in human breast cancer growth, and the associated consequences of circadian disruption by exposure to light at night (LEN). The anti-cancer actions of the circadian melatonin signal in human breast cancer cell lines and
receptor
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that appe
actions that involve multiple pathways, including inhibition of p38 MAPK and repression of epithelial–mesenchymal transition (EMT). Studies have demonstrated that melatonin promotes genomic stability by inhibiting the expression of LINE-1 retrotransposons. Finally, research in animal and human models has indicated that LEN-induced disruption of the circadian nocturnal melatonin signal promotes the growth, metabolism, and signaling of human breast cancer and drives breast tumors to endocrine and chemotherapeutic resistance. These data provide the strongest understanding and support of the mechanisms that underpin the epidemiologic demonstration of elevated breast cancer risk in night-shift workers and other individuals who are increasingly exposed to LEN.

Key Words
- melatonin
- breast cancer
- nuclear receptors

Finally, research in animal and human models has indicated that LEN-induced disruption of the circadian nocturnal melatonin signal promotes the growth, metabolism, and signaling of human breast cancer and drives breast tumors to endocrine and chemotherapeutic resistance.

Endocrine-Related Cancer

Endocrine-Related Cancer (2015) **22**, R183–R204

Oncotarget
Open Access Impact Journal

Oncotarget. 2017 Jun 13; 8(24): 39896–39921.
Published online 2017 Mar 18. doi: 10.18632/oncotarget.16379

PMCID: PMC5503661
PMID: 28415828

Melatonin for the prevention and treatment of cancer

Ya Li,[1] Sha Li,[#2] Yue Zhou,[1] Xiao Meng,[1] Jiao-Jiao Zhang,[1] Dong-Ping Xu,[1] and Hua-Bin Li[#1,3]

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

Abstract

Go to:

The epidemio
of tumors. Be
some human t
activity, modu
survival signaling and tumor metabolism, inhibition on angiogenesis, metastasis, and induction of epigenetic alteration. Melatonin could also be utilized as adjuvant of cancer therapies, through reinforcing the therapeutic effects and reducing the side effects of chemotherapies or radiation. Melatonin could be an excellent candidate for the prevention and treatment of several cancers, such as breast cancer, prostate cancer, gastric cancer and colorectal cancer. This review summarized the anticancer efficacy of melatonin, based on the results of epidemiological,experimental and clinical studies, and special attention was paid to the mechanisms of action.

"…different mechanisms of melatonin's anticancer effects were identified successively, such as inducing apoptosis, antiestrogenic effect through ERα-signaling pathway and inhibiting aromatase activity, modulation of melatonin receptors, and inhibition on invasion, and angiogenesis."

cancers

MDPI

Review

A New Paradigm in the Relationship between Melatonin and Breast Cancer: Gut Microbiota Identified as a Potential Regulatory Agent

Aurora Laborda-Illanes [1,2], Lidia Sánchez-Alcoholado [1,2], Soukaina Boutriq [1,2], Isaac Plaza-Andrades [1], Jesús Peralta-Linero [1], Emilio Alba [1,3,*], Alicia González-González [1,*] and María Isabel Queipo-Ortuño [1,3]

1 Unidad de Gestión Clínica Intercentros de Oncología Médica, Hospitales Universitarios Regional y Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga (IBIMA)-CIMES-UMA, 29010 Málaga, Spain; aurora.laborda@ibima.eu (A.L.-I.); l.sanchez.alcoholado@ibima.eu (L.S.-A.); soukaina@ibima.eu (S.B.); isaac.plaza.andrades@ibima.eu (I.P.-A.); jesus.peralta@ibima.eu (J.P.-L.); maribel.queipo@ibima.eu (M.I.Q.-O.)
2 Facultad de Medicina, Universidad de Málaga, 29071 Málaga, Spain
3 Centro de Investigación Biomédica en Red de Cáncer (Ciberonc CB16/12/00481), 28029 Madrid, Spain
* Correspondence: ealbac@uma.es (E.A.); alicia.gonzalez@ibima.eu (A.G.-G.)

Simple Summary: The relationship between melatonin and breast cancer has been widely described. On the other hand, in recent years, an imbalance in the composition of the intestinal bacterial population has been linked as another possible trigger for this disease. Given that changes in the gut microbiota have been observed to stimulate the kinurenine pathway, reducing circulating melatonin levels, in this review, we summarize the relationship between circadian disruption and breast cancer, as well as the connection with dysbiosis as possible causing this pathology due to a series of changes that lead to an increase in circulating estrogen levels.

Abstract: In this review we summarize a possible connection between gut microbiota, melatonin production, and breast cancer. An imbalance in gut bacterial population composition (dysbiosis), or changes in the production of melatonin (circadian disruption) alters estrogen levels. On the one hand, this may be due to the bacterial composition of estrobolome, since bacteria with β-glucuronidase activity favour estrogens in a deconjugated state, which may ultimately lead to pathologies, including breast cancer. On the other hand, it has been shown that these changes in intestinal microbiota stimulate the kynurenine pathway, moving tryptophan away from the melatonergic pathway, thereby reducing circulating melatonin levels. Due to the fact that melatonin has antiestrogenic properties, it affects active and inactive estrogen levels. These changes increase the risk of developing breast cancer. Additionally, melatonin stimulates the differentiation of preadipocytes into adipocytes, which have low estrogen levels due to the fact that adipocytes do not express aromatase. Consequently, melatonin also reduces the risk of breast cancer. However, more studies are needed to determine the relationship between microbiota, melatonin, and breast cancer, in addition to clinical trials to confirm the sensitizing effects of melatonin to chemotherapy and radiotherapy, and its ability to ameliorate or prevent the side effects of these therapies.

check for updates

Citation: Laborda-Illanes, A.; Sánchez-Alcoholado, L.; Boutriq, S.; Plaza-Andrades, I.; Peralta-Linero, J.; Alba, E.; González-González, A.; Queipo-Ortuño, M.I. A New Paradigm in the Relationship between Melatonin and Breast Cancer: Gut Microbiota Identified as a Potential Regulatory Agent. *Cancers* **2021**, *13*, 3141. https://doi.org/10.3390/cancers13133141

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Published: 23 June 2021

Suppresses Melatonin?

- Caffeine
- Tobacco and alcohol
- White and blue-light exposure at night (ie. phones)
- Variants in snps AANAT and ASMT
- Medications: Beta Blockers, NSAIDS

- Murphy PJ, Myers BL, Badia P. Nonsteroidal anti-inflammatory drugs alter body temperature and suppress melatonin in humans Physiology & Behavior. 1996; 59(1):133-139.
- https://www.stlukes-stl.com/health-content/medicine/33/000970.htm

Increasing Melatonin

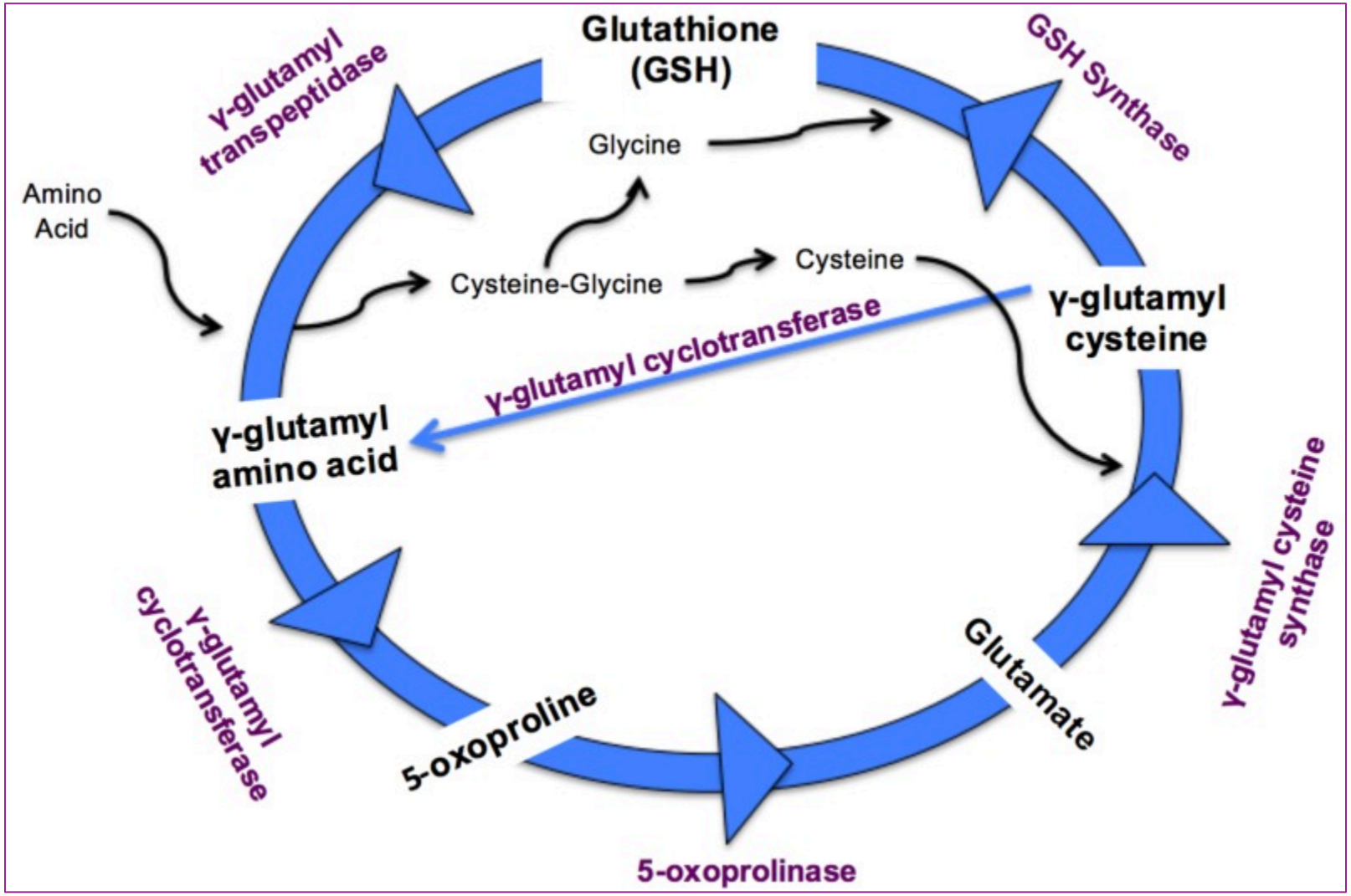
- See the **prior slide**
- Improve the **circadian rhythm** (see cortisol slides)
- Improve **gut health**
- Consider **snp testing** for melatonin if suspected
- Consider **melatonin supplementation**
  - Physiologic is 0.5mg, oncology often uses 10mg or higher
  - Foods, like pistachios and tart cherries contain melatonin

Key DUTCH Markers and Breast Cancer

1. **Estrogen excess**
2. **Phase 1 metabolites: 4-OH-E1 and 16-OH-E1**
3. **Phase 2 metabolite: methylation**
4. **Cortisol Pattern**
5. **Melatonin**
6. **Pyroglutamate**
7. 8OHdG

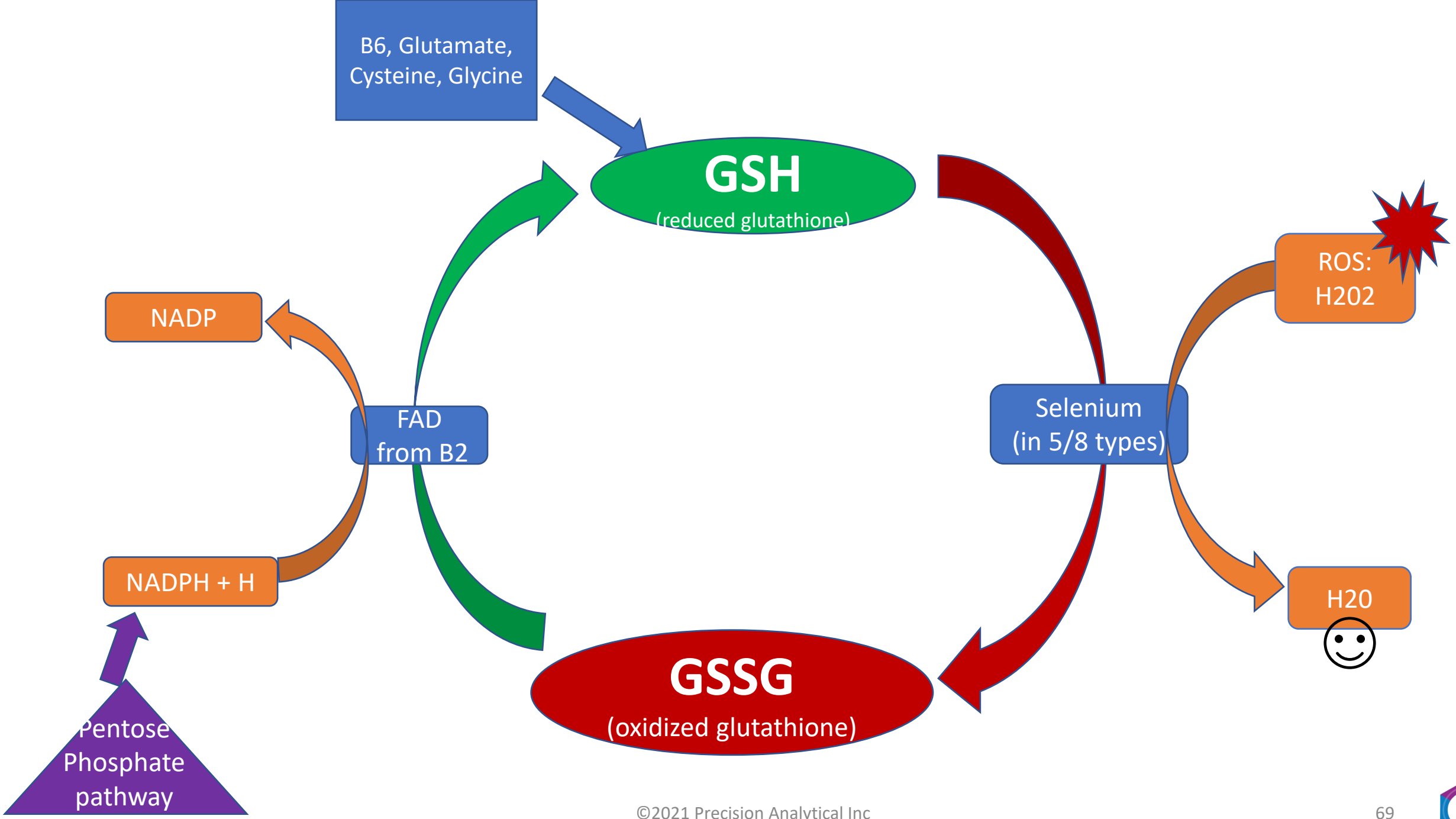
Pyroglutamate = Pyroglutamic acid = 5-Oxoproline = Metabolite in glutathione cycle

Glutathione Marker (may be deficient if low or high) - (Urine)				
Pyroglutamate	High end of range	55.9	ug/mg	32 - 60



Venkataraman SS, Regone R, Ammar HM, Govindu RR. Pyroglutamic Acidemia: An Underrecognized and Underdiagnosed Cause of High Anion Gap Metabolic Acidosis - A Case Report and Review of Literature . 2019; Cureus. Jul; 11(7): e5229.

How Does Glutathione Work?



Are all ROS bad?

- It's a balance
- Remember they form from normal bodily functions too such as eating, digesting, exercising, and creating ATP.
- ROS act as signaling molecules to the immune system.
- They can be toxic to tumors too, but this is controversial.

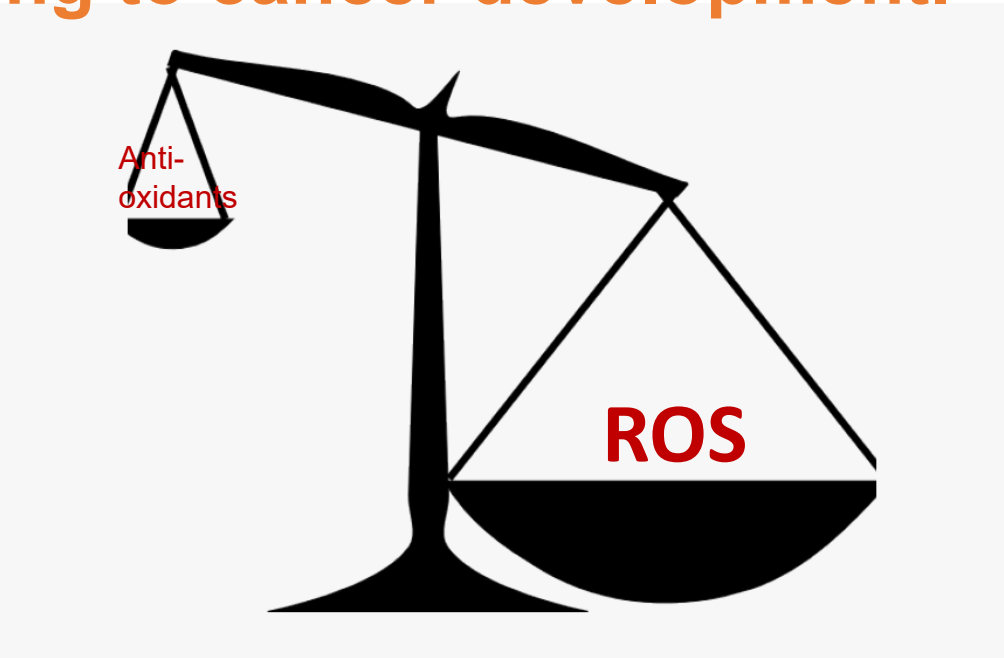
ROS and Breast Cancer

- "…oxidative stress **changed the redox potential** of the breast, leading to **drastic changes** in the DNA base lesions, which are conducive to oxidative conditions and breast cancer formation."
  - Zhang, ML., Wu, HT., Chen, WJ. *et al.* Involvement of glutathione peroxidases in the occurrence and development of breast cancers. *J Transl Med* **18,** 247 (2020). https://doi.org/10.1186/s12967-020-02420-x

ROS and Breast Cancer

- If glutathione is low, this could allow the ROS balance to become

UNbalanced leading to cancer development.



- Zhang, ML., Wu, HT., Chen, WJ. *et al.* Involvement of glutathione peroxidases in the occurrence and development of breast cancers. *J Transl Med* **18,** 247 (2020). https://doi.org/10.1186/s12967-020-02420-x

Advanced Glycation End Products (AGEs)

- Proteins and lipids (fats) that go **through glycation when exposed to sugars**
- As they advance, they are associated with **more inflammation, oxidative stress, and disease development**
- Increased in and associated with **breast cancer**
- Glutathione helps **reduce the AGEs effect** on the body

- Omofuma OO, Turner DP, Peterson LL, Merchant AT, Zhang J, Steck SE. Dietary Advanced Glycation End-products (AGE) and Risk of Breast Cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) Cancer Prev Res. 2020; 13(7):601-610.
- Sharma AK, Sharma VR, Gupta GK, Ashraf GM, Kamal MA. Advanced Glycation End Products (AGEs), Glutathione and Breast Cancer: Factors, Mechanism and Therapeutic Interventions CDM. 2019; 20(1):65-71.

Improving Glutathione

- Address the **cause(s)** excessively needing glutathione's powers
- Increase **precursors** such as N-Acetyl-Cysteine and B6
- Increase **co-factors** such as selenium and B2
- Increase **other antioxidants** that help recycle glutathione such as ascorbic acid and vitamin E

Key DUTCH Markers and Breast Cancer

1. Estrogen excess
2. Phase 1 metabolites: 4-OH-E1 and 16-OH-E1
3. Phase 2 metabolite: methylation
4. Cortisol Pattern
5. Melatonin
6. Pyroglutamate
7. 8OHdG

8-hydroxy-2-deoxyguanosine (8-OHdG)

Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)				
8-OHdG (Waking)	Above range	8.1	ng/mg	0 - 5.2

8-hydroxy-2-deoxyguanosine (8-OHdG)

- A sensitive but **not reason-specific** maker used for estimating **DNA damage due to oxidative stress** (ROS creation)

8-hydroxy-2-deoxyguanosine (8-OHdG)

- A sensitive but **not reason-specific** maker used for estimating **DNA damage due to oxidative stress** (ROS creation)
- Considered **pro-mutagenic** as it's a biomarker for various cancers and degenerative disease initiation and promotion

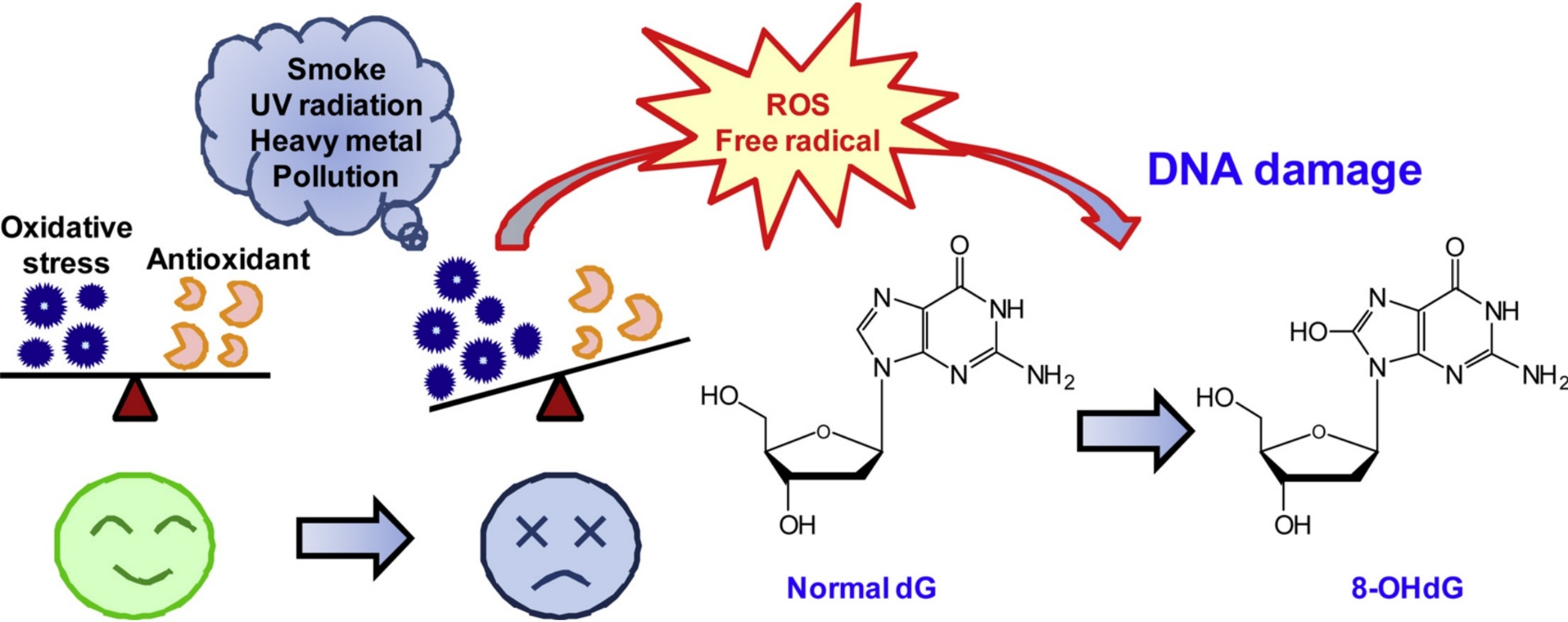
8-hydroxy-2-deoxyguanosine (8-OHdG)

- A sensitive but **not reason-specific** maker used for estimating **DNA damage due to oxidative stress** (ROS creation)
- Considered **pro-mutagenic** as it's a biomarker for various cancers and degenerative disease initiation and promotion
- Used to estimate **DNA damage after exposure to cancer-causing agents**: tobacco smoke, asbestos fibers, ROS, heavy metals, benzene, radon, arsenic, Nickle, toluene, toluene, and polycyclic aromatic hydrocarbons

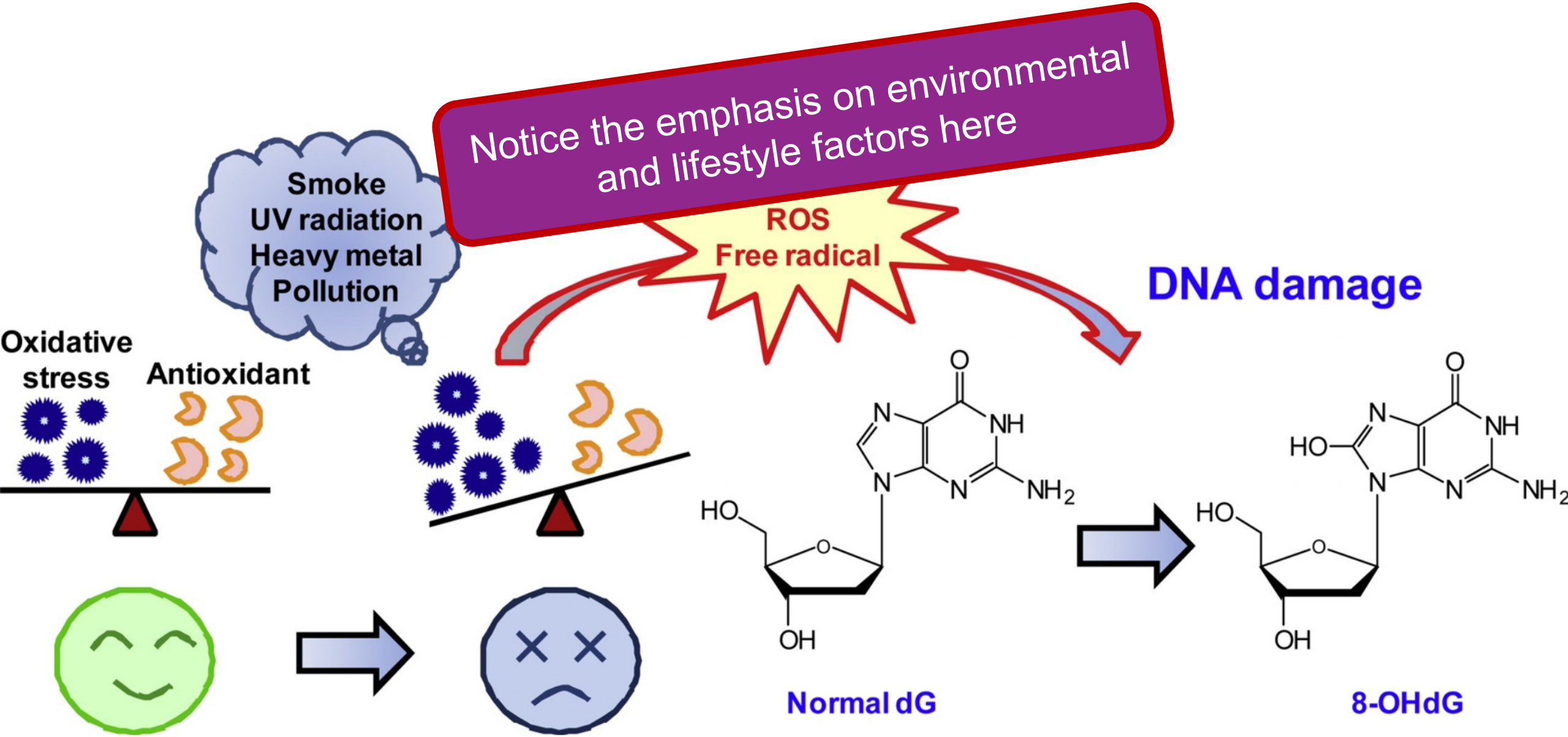
8-hydroxy-2-deoxyguanosine (8-OHdG)

- When local antioxidant systems fail, **oxidative damage permanently occurs** to lipids of cellular membranes, proteins and DNA
- **ROS attack Guanine** bases easily forming 8-OHdG
- 8-OHdG **binds to thymidine** rather than cytosine so it's a G→T transversion
- This is the most frequent **somatic mutations** (non germ cell) found in cancers
- These get removed by DNA repair mechanisms and it **shows up in urine**

- VALAVANIDIS A, VLACHOGIANNI T, FIOTAKIS C. 8-hydroxy-2′ -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis Journal of Environmental Science and Health, Part C. 2009; 27(2):120-139.
- Ock C. 8-Hydroxydeoxyguanosine: Not mere biomarker for oxidative stress, but remedy for oxidative stress-implicated gastrointestinal diseases WJG. 2012; 18(4):302-.



Kataoka H, Mizuno K, Oda E, Saito A. Determination of the oxidative stress biomarker urinary 8-hydroxy-2-deoxyguanosine by automated on-line in-tube solid-phase microextraction coupled with liquid chromatography tandem mass spectrometry Journal of Chromatography B. 2016; 1019:140-146.



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Original Study

Check for updates

8-Hydroxy-2'-deoxyguanosine as a Discriminatory Biomarker for Early Detection of Breast Cancer

Essam Eldin Mohamed Nour Eldin,[1] Mahmoud Zaki El-Readi,[1,2]
Mohamed Mahmoud Nour Eldein,[1,3] Albagir Ali Alfalki,[4]
Mohammad Ahmad Althubiti,[1] Hala Fawzy Mohamed Kamel,[1,3]
Safaa Yehia Eid,[1] Hiba Saeed Al-Amodi,[1] Ahmad A. Mirza[5]

Abstract

For early detection of malignant tumors, serum levels of 8-hydroxy-2'-deoxyguanosine were determined in 50 women with benign breast tumors, 50 women with breast cancer (BC), and 50 healthy women as a control group. 8-hydroxy-2'-deoxyguanosine levels were significantly increased in the BC group compared with the benign tumor and the healthy control groups; thus it can be used as a potential noninvasive biomarker for early detection of BC.

Background: Breast cancer (BC) is one of the most prevalent and reported cancers among Saudi women. Detection of BC in the early invasive stage (stages I, II) has an advantage in treating patients over detection in the late invasive stage (stages III, IV). Tumor markers are used to aid in diagnosis, treatment monitoring, and recurrence detection of malignant tumors. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a marker of nucleic damage owing to oxidative stress. **Patients and Methods:** We studied the blood levels of 8-OHdG in 50 women with benign breast tumors, 50 women with BC, and 50 healthy women as a control group. **Results:** The concentrations of 8-OHdG were significantly increased in the BC group (55.2 ng/dL) compared with the benign tumor group (30.2 ng/dL) and with the healthy control group (9.08 ng/dL). The same pattern was observed with other diagnostic markers, including carcinoembryonic antigen and cancer antigen 15-3. Significant positive correlations between 8-OHdG and both carcinoembryonic antigen ($r = 0.63$; $P < .001$) and cancer antigen 15-3 ($r = 0.51$; $P < .001$) were noticed. The levels of 8-OHdG were significantly higher in stage I (81 ng/dL) compared with stage II (51 ng/dL; $P < .05$), stage III (38 ng/dL; $P < .01$), and stage IV (19 ng/dL; $P < .001$). In addition, serum 8-OHdG had a high diagnostic performance in BC (area under the curve, 0.86; sensitivity = 82%; specificity = 80% at cutoff value 21.4 ng/mL). 8-OHdG is associated with BC risk according to logistic regression analysis. **Conclusion:** We concluded that the significant increase of serum levels of 8-OHdG in patients with BC can be used as a potential noninvasive biomarker for early detection of BC. However, large sample sizes from different stages and types of BC should be included in any future study to confirm the present findings before translating the findings into routine clinical application.

Clinical Breast Cancer, Vol. 19, No. 2, e385-93
Keywords: Antioxidants, Diagnostic tools, DNA, Neoplasm, Oxidative damage

Improving 8-OHdG

- Address the **cause(s)** as best you can
- Update **breast imaging** if warranted/needed
- Evaluate for **antioxidants**
  - Examples: Melatonin and pyroglutamate on the DUTCH test
- Consider **improving antioxidant status** through diet/supplementation if warranted

VALAVANIDIS A, VLACHOGIANNI T, FIOTAKIS C. 8-hydroxy-2′ -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis Journal of Environmental Science and Health, Part C. 2009; 27(2):120-139.

In Summary:

- Breast cancer is **complicated** – don't just blame estrogen.
- The 2 biggest risks are being female and aging HOWEVER it is an **unfortunate combination of genetics, hormones, lifestyle, and environmental factors.**
- **Prevention** and risk reduction are the goals!
- **Use the DUTCH test** and those 7 markers as one of the prevention tools in your toolbox.

…and that concludes our talk

Thank you for listening.

Lecture questions?
info@dutchtest.com

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