Its Not The Hormone, But Its Home;
Oxidative Stress, the Lens that Bends Health
Cheryl Burdette, ND
Review Of Hormones

I. Current Use Hormones
   A. Standard of Care
   B. A Functional Medicine Approach

II. Safety of Hormones
   A. Oxidative and Inflammatory Roles
   B. Hormones as An Axis: Adrenals, Thyroid, Reproductive

III. Protocols with Estrogens and Phytoestrogens
The Need

• Do No Harm—a healthy system is the backdrop for safe hormone use
• Menopausal Symptom Relief
• Get Side Benefits
  • Coagulation
  • Bone Health
  • Anti-inflammatory
  • Anti-Oxidant
  • Cognitive
  • Cardiovascular
How to Treat Hormone Imbalance

- Take a History; Do an Exam; Test as needed; Look for Cause
- Treat the Matrix:
  - Brain
  - GUT
  - Detoxification Capacity
  - Mitochondrial Function/Oxidative Stress
  - Immune Balance
  - Structural Imbalance
  - Mind–Body–Spirit
  - Hormone Imbalance
- THEN Treat Symptoms – OR Treat Symptoms WHILE searching for and ameliorating causes.
Relief for hot flushes, a predominant symptom of menopause is among the most common reasons for clinical visits of mid-life women and a major cost in health care expenditures. The median duration of moderate to severe hot flushes was 10.2 years...The most common ages at onset of moderate-to-severe hot flushes were 45–49 years.

Hot flushes are associated with poor sleep, depressed mood, decreased quality of life, may worsen depressive symptoms and signal the onset or relapse of a major depressive episode.

Hot flushes may possibly mark underlying vascular changes that are associated with subclinical cardiovascular disease, increased aortic calcification among users of hormone therapy, greater incident coronary heart disease, and may be a risk factor for poor bone health.
“Hot flushes may possibly mark underlying vascular changes that are associated with subclinical cardiovascular disease, increased aortic calcification among users of hormone therapy, greater incident coronary heart disease, and may be a risk factor for poor bone health.”
Prevalence of Hot Flashes

- 6.5% in Premenopause
- 56.6% in Perimenopause
- 50.7% in Postmenopause

Prevalence of Insomnia

- 14% in Premenopause
- 79% in Perimenopause
- 39% in Postmenopause

Background: Because hot flashes can occur during the night, their presence has been frequently associated with insomnia in women with symptoms of menopause. However, many factors other than hot flashes or menopause can be responsible for insomnia, and several factors associated with insomnia in the general population are also commonly observed in perimenopausal and postmenopausal women who have hot flashes.

Methods: A random sample of 3243 subjects (aged ≥18 years) representative of the California population was interviewed by telephone. Included were 982 women aged 35 to 65 years. Women were divided into 3 groups according to menopausal status: premenopause (57.2%), perimenopause (22.3%), and postmenopause (20.5%). Hot flashes were counted if they were present for at least 3 days per week during the last month and were classified as mild, moderate, or severe according to their effect on daily functioning. Chronic insomnia was defined as global sleep dissatisfaction, difficulty initiating sleep, difficulty maintaining sleep, or nonrestorative sleep, for at least 6 months. Diagnoses of insomnia were assessed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV).

Results: Prevalence of hot flashes was 12.5% in premenopause, 79.0% in perimenopause, and 39.3% in postmenopause. Prevalence of chronic insomnia was reported as 36.5% in premenopause, 56.6% in perimenopause, and 50.7% in postmenopause (p<.001). Prevalence of symptoms of chronic insomnia increased with the severity of hot flashes, reaching more than 80% in perimenopausal women and postmenopausal women who had severe hot flashes. In multivariate analyses, severe hot flashes were significantly associated with symptoms and a diagnosis of chronic insomnia. Poor health, chronic pain, and sleep apnea were other significant factors associated with chronic insomnia.

Conclusions: Severe hot flashes are strongly associated with chronic insomnia in midlife women. The presence of hot flashes should be systematically investigated in women with insomnia. Treating hot flashes could improve sleep quality and minimize the deleterious consequences of chronic insomnia.

Arch Intern Med. 2006;166:1262-1268
Menopausal Symptoms

- Hot flashes
- Fatigue
- Memory fog
- Aches and Pains
- Insomnia
- Decreased Sex Drive
- Vaginal Dryness
- Mood Swings
- Depression
- Anxiety
What is your Risk and Benefit?

- Family history of Alzheimer's
- Family History of Osteoporosis
- Family History of Heart Disease
- Family History of Breast Cancer
- Family History of Auto-Immune Disease
Premarin

- Comes From Horse Urine
- Contains 14 different types of estrogen
- Our body makes 3 different types of estrogen
- Contains no progesterone, testosterone or DHEA

Premarin is a mixture of 30-plus substances derived from the urine of pregnant mares. Estradiol — the dominant sex-steroid hormone in woman — accounts for about 17 percent of Premarin’s total content.
Not all Estrogens are the Same

- Estradiol—the most powerful estrogen that reduces hot flashes, improves bone density, may cause increase division of tissue
- Estriol—High in pregnancy, protective from cancer, not well measured in the serum
- Estrone—Weak estrogen, increases with menopause
- Catechol estrogens—Metabolites that may be beneficial or harmful depending on type and tissue
- Quinone estrogens—Metabolites most responsible for forming adducts with DNA
Benefits of Estradiol

- Estradiol is the most active of the estrogens responsible for actions attributed to the estrogens.
- Estriol has an intermediate activity and estrone is the least active of the estrogens.
- Estrone increase with menopause and is mostly derived from androstenedione, which is secreted by the adrenal cortex.
- Estradiol plays a role in immune activation, and potentially over activation.
- Higher levels of estradiol have been found to stimulate an immune response which is suppressed by androgens.
- Also Estradiol possesses neuroprotective and antiapoptotic properties as an NMDA antagonist. NMDA has been found to be a neurotoxin increasing neuronal apoptosis and necrosis.
- When cultures were incubated with estradiol and estriol, there was less cell death.
Estriol actually had more of a protective effective in the brain, even though estradiol binds more tightly to the estrogen receptor.

Neurogenic inflammation is also reduced by estradiol decreasing bladder disorders.

Appropriate levels of estradiol reduce reactive oxygen species and increases coronary blood flow.

However when estradiol levels are too high or unopposed not only can they increase the risk of breast AND prostate cancer.
Estrogen Metabolite Ratios: Time for Us to Let Go

by Jacob Schor, ND, FABNO
2–OHestrone

- 2–alpha Hydroxyestrone is more protective, with antiproliferative properties. [13]
- 2–alpha Hydroxyestrone has also been found to decrease lipid peroxidation of neuronal tissue, offering protection to the CNS. [14]
- Not only does it modulate estrogen activity by blocking more stimulating forms of estrogen, it interacts directly with the DNA, increasing expression of a gene that is involved in apoptosis.
- 2–Hydroxyestrone also has antiangiogenic effects as well as inhibiting mitotic progression through disruption of spindle formation. [15]
**2:16 Hydroxyestone Ratio with Bone Resorption: Profile**

<table>
<thead>
<tr>
<th></th>
<th>Patient Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:16 Ratio</td>
<td>1.9</td>
<td>2.0-19.5</td>
</tr>
<tr>
<td>Bone Resorption (NTx)</td>
<td>6.2</td>
<td>≤83.0*</td>
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**Nanomoles of cross linked N-teleopeptide (NTx) of Type I collagen per mg of total urinary protein.**
Lifestyle factors also influence 2 hydroxylation or estrone. Both exercise and a high protein diet were found to improve ratios. [23, 24]

Even though a large body of research exists demonstrating safety with I3C, more recent studies may begin to favor DIM, a derivative of I3C because it is not dependent on acidification in the gut and it does not increase 4OH of estrone.
DIM (3,3’-diindolylmethane) is the stable, bioactive metabolite formed when stomach acid breaks down indole-3-carbinol (I3C), a sulfur-containing glucosinolate present in cruciferous vegetables. [Bradlow HL 2008]

Supplementation with DIM is preferred over I3C due to I3C’s undesirable breakdown products, including the dioxin-like molecule indolo[3,2-b]carbazole (ICZ), [Herrmann S, et al. 2002]

DIM has been found to support hormone metabolism, stimulate antioxidant and detoxification systems, and possess immune-enhancing properties. [Ribly JE, 2006] Curcumin and BioPerine provide synergistic support for DIM’s role in supporting healthy cell metabolism.
There is some recent evidence that suggests that Indole-3-carbinole may increase 4-hydroxylation of estrone.

4-hydroxylation has been demonstrated to promote breast and prostate cancer tissue. [Biochim Biophys Acta. 2006 Apr 19;]

However, 4OH estrone is an extremely minor metabolite, comprising less than 1% of estrogen metabolism.
DIM Improves Estrogen and Testosterone

**Support of Hormone Metabolism** DIM promotes metabolism of estrogen into the favorable and protective 2-hydroxyestrogen (2-OHE) metabolites, reducing production of DNA-damaging 4-hydroxyestrogen (4-OHE) and 16-alpha-hydroxyestrogen (16-alpha-OHE) metabolites [Cavalieri E 2000].

DIM’s influence on 2-OHE production creates a more desirable ratio of 2-OHE to 16-alpha-OH. Assessment of 2:16-alpha-OHE ratio appears to be useful in assessing breast health. [Im A 2009] DIM also has been studied for its role in supporting prostate health as well, by reducing dihydrotestosterone binding to androgen receptors.

Research indicates that DIM has been found to have considerable antiandrogenic and antiproliferative effects [Fares F, 2010], [Le HT 2003]

Thyroid gland is also dependent on hormone balance. DIM was found to target proteolytic enzymes (MMP-2 and MMP-9), thus reducing unfavorable migration, adhesion, and invasion of thyroid cells in vitro. (Rajoria S PLoS One 2011)

Down-regulation of deleterious proteins (survivin, Bcl-2, and cdc25A) [Ahmad A 2010] and up-regulation of protective proteins (NRF2 and cyclin-dependent kinase inhibitor p21waf1) promoted healthy cell growth. [Rahman KW 2006].
Estrogen Metabolism

- Catechols (2-OH, 4-OH, 16-OH)
- Quinones
- Estrone
- Estriol
- Estradiol
- ---- Context changes activity
Hormone Metabolism

Cholesterol
  \[ \text{CYP11A} \]
  \[ \text{Pregnenolone} \]
  \[ \text{CYP17} \]
  \[ \text{Androstenedione} \]
  \[ \text{CYP19} \]
  \[ \text{Estrone} \]
  \[ \text{Sulfotransferase} \]
  \[ \text{Steroid sulfatase} \]
  \[ \text{Estrone sulfate} \]

Testosterone
  \[ \text{CYP19} \]
  \[ \text{Estradiol} \]

17β-HSD

2-Hydroxyestradiol
  \[ \text{CYP1A1/2} \]
  \[ \text{CYP3A4} \]

4-Hydroxyestradiol
  \[ \text{CYPs} \]
  \[ 6\alpha-, 6\beta-, 7\alpha-, 12\beta-, 15\alpha-, 15\beta-, 16\alpha-, 16\beta- \]

2-Methoxyestradiol
  \[ \text{COMT} \]

17β-HSD

Estradiol-2,3-semiquinone

Estradiol-2,3-quinone

Peroxidases

Estradiol-3,4-semiquinone

Estradiol-3,4-quinone

DNA damage
  \[ \text{Genotoxic carcinogenesis} \]

Inhibition of tumor
  \[ \text{Non-carcinogenesis} \]

Estrogen receptor binding
  \[ \text{Transcriptional activation} \]
Oxidative Stress

Positive Correlation with Chronic Disease and Aging

- Atherosclerosis
- Blood Vessels
- Brain
- Heart
- Joints
- Lungs
- Eyes
- Skin
- Dermatitis
- Psoriasis
- Scleroderma
- Cataracts
- Macular degeneration
- Alzheimer’s
- Parkinson’s
- Epilepsy
- Stroke
- Heart Attack
- Multiple Organs
  - Diabetes
  - Cancer
  - Inflammation
  - Aging

Figure from Evans, JL et al (2000) Diabetes Technol Therap 2:401–413.
Is It Estrogen, Or Estrogen’s Home

- What Determines Whether Estrogen is Good Or Bad
- What is Estrogen Really Doing?
  -----Upregulation of Glutathione Peroxidase
  -----Upregulation of Zonulin occludens
Biomarkers that help track oxidation relevant to estrogens and hormones

- % reduced glutathione
- F2-Isoprostane
- **8-OHdG**
- Glutathione Peroxidase
- SOD in Osteoporosis and Aging
Hormone therapy and oxidative stress

Oxidative stress measured by carbonyl groups level in postmenopausal women after oral and transdermal hormone therapy.
Polac I, Borowiecka M, Wilamowska A, Nowak P.
Source
Department of Gynaecology and Menopausal Disorders, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland. irekpolac@interia.pl
Menopause is associated with an increased risk of cardiovascular disorders, which are accompanied by oxidative stress. Our study was undertaken to determine whether oxidative stress in menopausal women could be reduced after six months of oral or transdermal hormonal therapy.
MATERIAL AND METHODS:
Carbonyl groups of proteins in blood plasma were estimated by sensitive ELISA method with anti-DNP antibodies. In this method, protein samples diluted in phosphate-buffered saline were adsorbed to wells of an ELISA plate and then reacted with dinitrophenylhydrazine (DNPH).
RESULTS:
Plasma protein carbonyl levels of postmenopausal women treated with o-HT and t-HT for six months (o-HT: 1.785 ± 0.31 nmol/mg; t-HT: 1.838 ± 0.33 nmol/mg) were lower when compared with the control group (2.232 ± 0.28 nmol/mg). There was no statistically significant difference in carbonyl levels between women after oral and transdermal HT (P = 0.149).
CONCLUSION:
Hormonal therapy reduces the level of carbonyl protein, a marker of oxidative stress, suggesting potential protective effect.
Hormone therapy may reduce oxidative damage

Escalante Gómez C, Quesada Mora S.

HRT decreases DNA and lipid oxidation in postmenopausal women.

Postmenopausal women have increased oxidative stress and decreased antioxidant status. Estrogen has great antioxidant capacity both in vitro and in vivo. Few authors have studied the effect that hormone replacement therapy (HRT) has on the oxidant and antioxidant status and none have studied the effect on DNA oxidation as a possible explanation for the aging process itself.

AIM:
The aim of this study was to evaluate both oxidation and antioxidation markers in postmenopausal woman and to determine the effects that HRT has on them.

METHOD:
Sixty-two postmenopausal women with similar biophysical characteristics were divided into three groups: (1) 18 not taking any HRT, (2) 20 receiving estrogen–only replacement therapy (ERT, conjugated equine estrogen), and (3) 22 receiving combined estrogen/progestin HRT (conjugated equine estrogen + medroxyprogesterone acetate). Specific molecular oxidative damage was detected by measuring 8– hydroxy–2–deoxy guanosine (8–OH–2dG) (DNA damage), standardized thiobarbituric acid reactive substances (TBARS) (lipid damage) and protein carbonyl (proteins). Antioxidant enzyme activity was detected by measuring catalase activity, and total antioxidant status was measured using 1,1,difenil–2–picril hydrazil. Both ELISA and photometric methods were used.

RESULTS:
8–OH–2dG levels were significantly lower in women who received combined HRT compared to women who did not receive HRT (ANOVA, p < 0.05). Lipid oxidation was significantly lower in women on ERT compared to women taking no HRT (ANOVA, p < 0.05). Pearson correlation showed that lipid oxidation decreased as the estradiol concentration increased within the study range (r = −0.362, p < 0.05). No statistical difference was noted for protein oxidation and catalase activity among the groups. No statistical difference was found for total antioxidant status between the groups (ANOVA).

CONCLUSIONS:
HRT decreases oxidative damage to both DNA and lipids in postmenopausal women. Lipid oxidation status may be inversely related to estrogen levels in postmenopausal women.
Recent human trial shows oxidative stress to be a key player in menopause


Menopause as risk factor for oxidative stress.

We carried out a cross-sectional study with 187 perimenopausal women from Mexico City, including 94 premenopausal (mean ± SD age, 44.9 ± 4.0 y; estrogen, 95.8 ± 65.7 pg/mL; follicle–stimulating hormone, 13.6 ± 16.9 mIU/mL) and 93 postmenopausal (mean ± SD age, 52.5 ± 3.3 y; estrogen, 12.8 ± 6.8 pg/mL; follicle–stimulating hormone, 51.4 ± 26.9 mIU/mL) women. We measured lipoperoxides using a thiobarbituric acid–reacting substance assay, erythrocyte superoxide dismutase and glutathione peroxidase activities, and the total antioxidant status with the Randox kit. An alternative cutoff value for lipoperoxide level of 0.320 μmol/L or higher was defined on the basis of the 90th percentile of young healthy participants. All women answered the Menopause Rating Scale, the Athens Insomnia Scale, and a structured questionnaire about pro-oxidant factors, that is, smoking, consumption of caffeinated and alcoholic beverages, and physical activity. Finally, we measured weight and height and calculated body mass index.

RESULTS:
The lipoperoxide levels were significantly higher in the postmenopausal group than in the premenopausal group (0.357 ± 0.05 vs 0.331 ± 0.05 μmol/L, P = 0.001). Using logistic regression to control pro-oxidant variables, we found that menopause was the main risk factor for oxidative stress (odds ratio, 2.62; 95% CI, 1.35–5.11; P < 0.01). We also found a positive correlation between menopause rating score, insomnia score, and lipoperoxides, and this relationship was most evident in the postmenopausal group (menopause scale, r = 0.327 [P = 0.001]; insomnia scale, r = 0.209 [P < 0.05]).

CONCLUSIONS:
Our findings suggest that the depletion of estrogen in postmenopause could cause oxidative stress in addition to the known symptoms.
Oxidation of estrogen may be an initiating tumor event


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

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–NAcCys] conjugates, as well as the methoxy CE, were identified and quantified by HPLC, whereas the 4–OHE1(E2)–1–N7Gua depurinating adducts and 4–OHE1(E2)–2–SG conjugates could only be identified by the mass spectrometry method. Most of the administered 4–OHE2 was metabolically converted to 4–OHE1. Formation of thioether (GSH, Cys and NAcCys) conjugates and depurinating adducts [4–OHE1(E2)–1–N7Gua] indicates that oxidation of 4–CE to CE–3,4–Q and subsequent reaction with GSH and DNA, respectively, do occur. The major conjugates in the urine were 4–OHE1(E2)–2–NACCYS: The oxidative pathway of 4–OHE1(E2) accounted for approximately twice the level of products compared with those from the methylation pathway. The metabolites and methoxy CE were excreted predominantly (>90%) as glucuronides, whereas the thioether conjugates were not further conjugated. These results provide strong evidence that exposure to 4–OHE1(E2) leads to the formation of E1(E2)–3,4–Q and, subsequently, depurinating DNA adducts. This process is a putative tumor initiating event. The estrogen metabolites, conjugates and adducts can be used as biomarkers for detecting enzymatic oxidation of estrogens to reactive electrophilic metabolites and possible susceptibility to estrogen–induced cancer.
Menopause can be defined as a condition of oxidative stress. Biomarkers of oxidative stress are higher post menopause. Studies also show that there is more oxidative stress in women who have more symptoms of menopause. Glutathione drops off in menopause which may be part of the reason for aging, nor merely decline in hormones. The implications clinically are important because this would mean we could treat menopause with glutathione therapies rather than hormones. This would be a safer and more benign method of treatment. Estrogens, even those that are bioidentical and taken exogenously are safer when in a high glutathione environment. That is to say, women on HRT should also consider glutathione therapies to reduce risk of HRT. This is a missed piece of hormone therapy. Glutathione therapies such as DIM and sulforaphane therapies should be considered as an adjunct for all HRT. It not only shifts a 2:16 ratio (of debatable importance), but increases glutathione which decreases 4-OH and favors production of 2-OH. Glutathione therapies should be considered alongside HRT.
Standard and modified paradigms for estrogen carcinogenesis.

Standard Paradigm
Estrogen, and perhaps progesterone “...affect the rate of cell division and thus manifest their effect on the risk of breast cancer by causing proliferation of breast epithelial cells. Proliferating cells are susceptible to genetic errors during DNA replication which, if uncorrected, can ultimately lead to a malignant phenotype.”
(Feigelson and Henderson, Carcinogenesis, 17:2279-84, 1996)

Modified Paradigm
While estrogen-induced cell proliferation undoubtedly has important role in the carcinogenic process, mounting evidence supports a complimentary pathway involving:
Indirect and direct genotoxicity originating from estrogen metabolites, i.e. 4-OHE₂
  • Indirect: Oxidative DNA damage via Redox Cycling → ROS
  • Direct: Estrogen-quinone DNA adducts
• Protective effects: Perhaps through 2-methoxy catechol estrogen-mediated growth inhibition, apoptosis and anti-angiogenesis
Effect of vitamin E and C supplements on lipid peroxidation and GSH–dependent antioxidant enzyme status in the blood of women consuming oral contraceptives.

Oral contraceptives (OCs) may affect oxidative stress status. We aimed to assess whether supplementation with vitamins E and C reduced this OC effect.

**STUDY DESIGN:** One hundred twenty healthy female individuals were divided into three groups: A, control; B, untreated OCU (OC users); and C, treated OCU (OC users with vitamin E and C supplementation). In all cases, plasma glutathione peroxidase (GPx) and glutathione reductase (GR) activities and malondialdehyde (MDA) level were determined.

**RESULTS:** Significant increases were found in the plasma MDA level, and activities of GPx and GR in plasma were decreased in Group B compared to the control group. Supplementation with vitamin C and E significantly increased the activity of GPx and GR activity, and reduced plasma MDA levels in Group C (p<.05).

**CONCLUSIONS:** These data suggest that low-dose OCs, by enhancing the stress oxidative and lipid peroxidation, may represent a potential cardiovascular risk factor, and the use of vitamins E and C may be beneficial in ameliorating this side effect of OCs.
Finally, we have shown that a major phase I metabolite of both equilenin and equilin (4-OHEN) can act as a complete carcinogen and tumor promoter \textit{in vitro}, whereas the endogenous catechol estrogen metabolite, 4-hydroxyestrone was much less effective.

Premarin To Quinones

EQUILIN \[\xrightarrow{\text{P450}}\] 4-OHEQ \[\xrightarrow{[\text{O}]}\] 4-OHEQ-\text{o-QUINONE}

EQUILENIN \[\xrightarrow{\text{P450}}\] 4-OHEN \[\xrightarrow{[\text{O}]}\] 4-OHEN-\text{o-QUINONE}

1. ISOMERIZE
2. [O]
Menopause: a review on the role of oxygen stress and favorable effects of dietary antioxidants.
Miquel J, Ramírez-Boscá A, Ramírez-Bosca JV, Alperi JD.

Abstract
Menopause is often accompanied by hot flashes and degenerative processes such as arteriosclerosis and atrophic changes of the skin that suggest an acceleration of aging triggered by estrogen lack. Therefore, hormone replacement therapy (HRT) has been considered the most suitable treatment for the above symptoms and processes. However, because of the possible serious side effects of HRT (especially the increased risk of thromboembolic accidents and breast cancer) there is a growing demand for alternative treatments of the symptoms and pathological processes associated with menopause. In agreement with the above, we review research that supports the concept that oxygen stress contributes to menopause and that some of its physiopathological effects may be prevented and/or treated improving the antioxidant defense of menopausic and postmenopausic women. Accordingly, a selection of micronutrients may be useful as a dietary supplement for protection against the decline of physiological functions caused by age-related oxygen stress. Since aging is accompanied by a progressive oxidation of the physiological sulfur pool, we emphasize the role of the vitamins B that help to maintain the GSH/GSSG ratio in its normal reduced state. Nutritional supplements should also include the key antioxidant vitamins C and E, as well as beta-carotene and the mineral micronutrients found in the oxygen radical-detoxifying enzymes glutathione peroxidase and superoxide dismutase. Moreover, the reviewed data support the concept that other antioxidants such as lipoic acid and the precursors of glutathione thioproline (TP) and 1–2–oxothiazolidine–4-carboxylic acid (OTC), as well as the soy isoflavones and the "coantioxidants" of an hydroalcoholic extract of Curcuma longa may help to prevent antioxidant deficiency with resulting protection of mitochondria against premature oxidative damage with loss of ATP synthesis and especialized cellular functions. Therefore, the administration under medical advice of synergistic combinations of some of the above mentioned antioxidants in the diet as well as topically (for skin protection) may have favorable effects on the health and quality of life of women, especially of those who cannot be treated with HR, suffer high levels of oxygen stress, and do not consume a healthy diet that includes five daily rations of fresh fruit and vegetables.
The effects of hormone replacement therapy on lipid peroxidation and antioxidant status.
Ozden S, Dildar K, Kadir YH, Gülizar K.
Source
Department of Biochemistry, Taksim State Hospital, Istanbul, Turkey.
Abstract
OBJECTIVE:
A number of studies have consistently shown a lower cardiovascular risk in women who received postmenopausal hormone replacement therapy (HRT). The aim of our study was to examine the effects of HRT on lipid peroxidation and antioxidant status, which were likely to be involved in the pathophysiology of atherosclerosis.
METHODS:
We measured erythrocyte and plasma thiobarbituric acid reactive substances (TBARS) levels as expression of lipid peroxidation–end product malondialdehyde, and also erythrocyte reduced glutathione (GSH) level and glutathione peroxidase (GSH–Px) activity as indicators of the antioxidant status of the 35 postmenopausal women with HRT (mean age: 51.81 +/– 4.57 yr; body mass index (BMI): 26.56 +/– 3.78 kg/m(2)) and 35 postmenopausal women without HRT (mean age: 47.50 +/– 3.64; BMI: 27.42 +/–3.43 kg/m2).
RESULTS:
In the group with HRT, erythrocyte and plasma TBARS levels were significantly lower than in the group without HRT (P < 0.003 and P < 0.001, respectively). Erythrocyte GSH level and GSH–Px activity was found to be increased significantly in the group with HRT in comparison with the group without HRT (P < 0.001 and P < 0.001, respectively). There was not any correlation between the erythrocyte and plasma TBARS and erythrocyte GSH levels and GSH–Px activity with duration of HRT (mean 3.5+/–1.3 yr).
CONCLUSION:
Our results show that HRT is beneficial in the protection against oxidative damage and can prevent atherosclerotic complications.

Covalent binding of catechol estrogens to glutathione catalyzed by horseradish peroxidase, lactoperoxidase, or rat liver microsomes.

Cao K, Devanesan PD, Ramanathan R, Gross ML, Rogan EG, Cavalieri EL.

Source
Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68198–6805, USA.

Abstract
Oxidation of catechol estrogens (CE) leads to the reactive electrophilic CE quinones. Reaction of CE-3,4-quinones with DNA has been implicated in tumor initiation. One pathway to prevent this reaction is conjugation of CE quinones with glutathione (GSH). Four CE, 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol (4-OHE2), 2-OHE1, and 2-OHE2, were conjugated with GSH after oxidation catalyzed by horseradish peroxidase (HRP), lactoperoxidase (LP), or rat liver microsomal cytochrome P450. This reaction is a free–radical chain autoxidation that produces very high yields of products. Six mono–GSH conjugates, 4-OHE1(E2)-2-SG, 2-OHE1(E2)-1-SG, and 2-OHE1(E2)-4-SG, and four di–GSH conjugates, 4-OHE1(E2)-1,2-bisSG and 2-OHE1(E2)-1,4-bisSG, were identified and quantified. These di–GSH conjugates were also obtained quantitatively from oxidation of mono–GSH conjugates by the same enzymes. HRP and LP gave very similar product profiles. Phenobarbital– and 3-methylcholanthrene–induced microsomes with either NADPH or cumene hydroperoxide as cofactor oxidized 4-OHE2 to form similar amounts of GSH conjugates. Enzymatic oxidation of 2-OHE1(E2) in the presence of GSH produced more 2-OHE1(E2)-4-SG than the 1-isomer. This contrasts with the direct reaction of E1(E2)-2,3-Q and GSH, in which the 1-isomer is formed more abundantly than the 4-isomer (Cao, K., Devanesan, P. D., Ramanathan, R., Gross, M. L., Rogan, E. G., and Cavalieri, E. L. (1998) Chem. Res. Toxicol. 11, 909–916). Competitive enzymatic oxidation of equimolar 4-OHE2 and 2-OHE2 in the presence of an equimolar amount of GSH yielded more 2-OHE2 conjugates than 4-OHE2 conjugates, despite E2-3,4-Q being more reactive with GSH than E2-2,3-Q. These results suggest that 2-OHE2 is a better substrate than 4-OHE2 in the catalytic oxidation to quinones, despite the greater reactivity of E2-3,4-Q, compared to E2-2,3-Q, with GSH.
Low Glutathione

- Methionine, NAC, Taurine, Sulfur
- Sulforaphane, Glucosinolates, DIM, Curcumin, Milk Thistle, Resveratrol
- Oral acetylated glutathione
- Topical glutathione
- ALA, Selenium,
Critical role of oxidative stress in estrogen–induced carcinogenesis

Mechanisms of estrogen–induced tumorigenesis in the target organ are not well understood. It has been suggested that oxidative stress resulting from metabolic activation of carcinogenic estrogens plays a critical role in estrogen–induced carcinogenesis. We tested this hypothesis by using an estrogen–induced hamster renal tumor model, a well established animal model of hormonal carcinogenesis. Hamsters were implanted with 17β–estradiol (βE2), 17α–estradiol (αE2), 17α–ethinylestradiol (αEE), menadione, a combination of αE2 and αEE, or a combination of αEE and menadione for 7 months. The group treated with βE2 developed target organ specific kidney tumors. The kidneys of hamsters treated with αE2, αEE, or menadione alone did not show any gross evidence of tumor. Kidneys of hamsters treated with a combination of αE2 and αEE showed early signs of proliferation in the interstitial cells. Kidneys of hamsters treated with a combination of menadione and αEE showed foci of tumor with congested tubules and atrophic glomeruli. βE2–treated tumor–bearing kidneys showed >2–fold increase in 8–iso–prostaglandin F$_{2\alpha}$ (8–iso–PGF$_{2\alpha}$) levels compared with untreated controls. Kidneys of hamsters treated with a combination of menadione and αEE showed increased 8–iso–PGF$_{2\alpha}$ levels compared with untreated controls, whereas no increase in 8–iso–PGF$_{2\alpha}$ was detected in kidneys of αEE–treated group. A chemical known to produce oxidative stress or a potent estrogen with poor ability to produce oxidative stress, were nontumorigenic in hamsters, when given as single agents, but induced renal tumors, when given together. Thus, these data provide evidence that oxidant stress plays a crucial role in estrogen–induced carcinogenesis.
High F2–Isoprostane

- Omega 3 fatty acids
- Vitamin E
- Green Tea
- CoQ10
- Glutathione therapies (above)
- Vitamin C
Arachidonic acid/F2-Isoprostane

ARACHIDONIC ACID

ENZYMATIC PATHWAY

FREE-RADICAL PATHWAY

PGF-2α Specific stereochemistry

F2-Isoprostanes, variable stereochemistry
Urinary F2–isoprostane provides a possible biomarker for the role for oxidative stress in prostate carcinogenesis.

Oxidative stress measured by urine F2–isoprostane level is associated with prostate cancer.

Barocas DA, Motley S, Cookson MS, Chang SS, Penson DF, Dai Q, Milne G, Roberts LJ 2nd, Morrow J, Concepcion RS, Smith JA Jr, Fowke JH.

Oxidative stress is implicated in prostate cancer by several lines of evidence. We studied the relationship between the level of F2–isoprostanes, a validated biomarker of oxidative stress, and prostate cancer and high grade prostatic intraepithelial neoplasia.

MATERIALS AND METHODS:
This case–control analysis within the Nashville Men's Health Study included men recruited at prostate biopsy. Body morphometrics, health history and urine were collected from more than 2,000 men before biopsy. F2–isoprostanes were measured by gas chromatography/mass spectrometry within an age matched sample of Nashville Men's Health Study participants that included 140 patients with high grade prostatic intraepithelial neoplasia, 160 biopsy negative controls and 200 prostate cancer cases. Multivariable linear and logistic regression was used to determine the associations between F2–isoprostane level, and high grade prostatic intraepithelial neoplasia and prostate cancer.

RESULTS:
Mean patient age was 66.9 years (SD 7.2) and 10.1% were nonwhite. Adjusted geometric mean F2–isoprostane levels were higher in patients with prostate cancer (1.82, 95% CI 1.66–2.00) or high grade prostatic intraepithelial neoplasia (1.82, 95% CI 1.68–1.96) than in controls (1.63, 95% CI 1.49–1.78, p <0.001), but were similar across Gleason scores (p = 0.511). The adjusted odds of high grade prostatic intraepithelial neoplasia and prostate cancer increased with increasing F2–isoprostane quartile (p-trend = 0.015 and 0.047, respectively) and the highest F2–isoprostane quartile was associated with significantly increased odds of prostate cancer (OR 2.44, 95% CI 1.17–5.09, p = 0.017).

CONCLUSIONS:
Pre–diagnosis urine F2–isoprostane level is increased in men with high grade prostatic intraepithelial neoplasia or prostate cancer, suggesting urinary F2–isoprostane provides a biomarker for the role for oxidative stress in prostate carcinogenesis. F2–isoprostanes may also serve to estimate the efficacy of interventions targeting oxidative stress mechanisms in prostate cancer prevention or treatment.
Transdermal estradiol reduces F2α-isoprostane levels in postmenopausal women.

Hermenegildo C, Oviedo PJ, Laguna A, García-Pérez MA, Tarín JJ, Cano A.

Source
Research Foundation, Hospital Clínico Universitario de Valencia, Valencia, Spain.

Abstract
OBJECTIVE:
F2α-isoprostanes are considered the most reliable index of in vivo oxidative stress. Given the implication of oxidative stress in the pathogenesis of atherosclerosis, we investigated the effects of hormone therapy on the plasma levels of F2α-isoprostanes.

DESIGN:
Sixty-one healthy postmenopausal women were treated in a randomized trial with estradiol either orally (2 mg/day, 28 women) or transdermally (50 mug/day, 33 women) for 4 weeks. Then women in each group were randomly assigned to oral progestogen, either micronized progesterone (300 mg/day) or medroxyprogesterone acetate (5 mg/day) for 2 additional weeks. Plasma samples were collected before and at the end of each treatment period, either estradiol alone or estradiol plus progestogen. F2α-isoprostanes were measured by a commercial enzyme immunoassay.

RESULTS:
A significant reduction in the levels of F2α-isoprostanes was detected only in women receiving transdermal estradiol, alone or in combination with medroxyprogesterone acetate.

CONCLUSIONS:
Transdermal estradiol alone or associated with medroxyprogesterone acetate decreased plasma levels of F2α-isoprostanes. These data elucidate additional details of the beneficial effect of estradiol on oxidative stress, a relevant mechanism in atherogenesis.
High 8-OHdG

- Green Tea
- Curcumin
- ALA

- Glutathione therapies (above)
- Resveratrol
Catechol estrogen and 8-OHdG as biomarkers of breast cancer risk


**Abstract**

There is association between exposure to estrogens and the development and progression of hormone–dependent gynecological cancers. Chemical carcinogenesis by catechol estrogens derived from oxidative metabolism is thought to contribute to breast cancer, yet exact mechanisms remain elusive. Malignant transformation was studied in MCF-10A human mammary epithelial cells, since estrogens are not proliferative in this cell line. The human and equine estrogen components of estrogen replacement therapy (ERT) and their catechol metabolites were studied, along with the influence of co–administration of selective estrogen receptor modulators (SERMs), raloxifene and desmethylarzoxifene (DMA), and histone deacetylase inhibitors. Transformation was induced by human estrogens, and selectively by the 4-OH catechol metabolite, and to a lesser extent by an equine estrogen metabolite. The observed estrogen–induced upregulation of CYP450 1B1 in estrogen receptor negative MCF-10A cells, was compatible with a causal role for 4-OH catechol estrogens, as was attenuated transformation by CYP450 inhibitors. Estrogen–induced malignant transformation was blocked by SERMs correlating with a reduction in formation of nucleobase catechol estrogen (NCE) adducts and formation of 8-oxo-dG. NCE adducts can be formed consequent to DNA abasic site formation, but NCE adducts were also observed on incubation of estrogen quinones with free nucleotides. These results suggest that NCE adducts may be a biomarker for cellular electrophilic stress, which together with 8-oxo-dG as a biomarker of oxidative stress correlate with malignant transformation induced by estrogen oxidative metabolites. The observed attenuation of transformation by SERMs correlated with these biomarkers and may also be of clinical significance in breast cancer chemoprevention.
Antioxidants, 8-OHdG and breast cancer, Reversal of the biomarker is meaningful


Superoxide dismutase 3 is induced by antioxidants, inhibits oxidative DNA damage and is associated with inhibition of estrogen–induced breast cancer.

Singh B, Bhat HK.

Source
Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri–Kansas City, 2464 Charlotte Street, Room 5251, Kansas City, MO 64108, USA.

Abstract
Epidemiological data and studies in rodent models strongly support the role of estrogens in the development of breast cancers. Oxidative stress has been implicated in this carcinogenic process. We have recently demonstrated that antioxidants vitamin C or butylated hydroxyanisole (BHA) severely inhibit 17β-estradiol (E2)–induced breast tumor development in female ACI rats. The objective of this study was to characterize the mechanism of antioxidant–mediated prevention of breast cancer. Female August Copenhagen Irish (ACI) rats were treated with E2, vitamin C, vitamin C + E2, BHA and BHA + E2 for up to 8 months. Superoxide dismutase 3 (SOD3) was suppressed in E2–exposed mammary tissues and in mammary tumors of rats treated with E2. This suppression was overcome by co–treatment of rats with E2 and vitamin C or BHA. 8–Hydroxydeoxyguanosine (8–OHdG) levels determined as a marker of oxidative DNA damage were higher in E2–exposed mammary tissues and in mammary tumors compared with age–matched controls. Vitamin C or BHA treatment significantly decreased E2–mediated increase in 8–OHdG levels in the mammary tissues and in MCF 10A cells. Increased DNA damage, colony and mammosphere formation, and migration in SOD3 knocked down MCF–10A cells, and nuclear translocation of SOD3 in vitamin C–treated mammary tissues and in MCF–10A cells suggest protective role of SOD3 against DNA damage and mammary carcinogenesis. Our studies further demonstrate that SOD3 is induced by antioxidants and is regulated through NRF2. SOD3 may thus be an important gene in defense against oxidative stress and in the prevention of estrogen–mediated breast cancer.
Oxidation and Methylation


Source
Department of Surgery, Keio University School of Medicine, Tokyo, Japan. matsui@mc.med.keio.ac.jp

Abstract
Reactive oxygen species (ROS) induced damage to DNA plays a major role in carcinogenesis. In order to estimate the level of oxidative damage and its role in breast cancer, 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined in DNA isolated from human breast tissue. Furthermore, we investigated whether polymorphisms in genes for enzymes involved in generation and elimination of ROS had any association with the level of 8-OHdG in breast tissue. In this study, the level of 8-OHdG in DNA was measured by the high performance liquid chromatography–electrochemical detector (HPLC–ECD) method. Genotypes of cytochrome P450 (CYP)1A1, glutathione S–transferase (GST)M1, GSTP1 and catechol O–methyltransferase (COMT) were determined by PCR–based restriction fragment length polymorphism analysis. A total of 61 Japanese patients were included in the study. The mean level of 8-OHdG in DNA of breast cancer tissues was 2.07 +/- 0.95 per 10^5 dG residues, while the mean level of 8-OHdG in DNA of non-cancerous breast tissues was 1.34 +/- 0.46 per 10^5 dG residues. The 8-OHdG levels in DNA of breast cancer tissues were significantly higher than those of their corresponding non-cancerous breast tissues (P < 0.0001). There was negative correlation between the clinical stage and the mean level of 8-OHdG in DNA of breast cancer tissues. Furthermore, patients with genotype of high GSTP1 activity had lower level of 8-OHdG in DNA of breast cancer tissues than others. On the contrary, the mean level of 8-OHdG in DNA of breast cancer tissues was higher among patients with genotype of high COMT activity. Our findings support the assumption that cancer cells are more exposed to oxidative stress than adjacent non-cancerous tissue. Genetic polymorphisms in enzymes involved in ROS metabolism may have a role in individual susceptibility to oxidant-related breast disease. At the same time, reduction of oxidative stress is thought to be a very important measure for primary prevention of breast cancer.

PMID: 10766427 [PubMed – indexed for MEDLINE]
Hormones

- High oxidative stress decreases methylation and increases quinone production
- If no shift in 4–OH estrone with 5–MTHF, think oxidative stress
Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis.

Musarrat J, Arezina–Wilson J, Wani AA.

Source
Department of Radiology, Ohio State University, Columbus 43210, USA.

Abstract
In order to estimate the level of oxidative damage and its role in breast cancer, the promutagenic oxidative lesion, 8-hydroxy-2'-deoxyguanosine (8-OHdG), was determined in DNA isolated from 75 human breast tissue specimens and from normal and transformed human breast cell lines, utilising a newly developed solid–phase immunoslot blot assay. The amount of 8-OHdG was found to be 0.25 +/- 0.03 pmol/microgram in normal breast tissue from reduction mammoplasty, 0.98 +/- 0.174 pmol/microgram in benign tumours and 2.44 +/- 0.49 pmol/microgram DNA in malignant breast tissue with invasive ductal carcinoma. The malignant tissue had a statistically significant 9.76-fold higher level of 8-OHdG than normal tissue (P < 0.001, Mann–Whitney). A statistically significant 12.9-fold (P = 0.004) higher endogenous formation of 8-OHdG was also observed in cultured breast cancers cells compared with normal breast epithelial cells. In addition, a significantly elevated level (3.35-fold higher, P < 0.05) of 8-OHdG observed in oestrogen receptor–positive compared with oestrogen–negative malignant tissues, and in breast cancer cell lines (9.3-fold higher, P = 0.007) suggests a positive relationship between 8-OHdG formation and oestrogen responsiveness. The extent of 8-OHdG adducts did not show a discernible correlation with either the age or the smoking status of the patients. These results indicate that the accumulation of 8-OHdG in DNA has a predictive significance for breast cancer risk assessment and is conceivably a major contributor in the development of breast neoplasia.
8–OHdG and breast cancer


_Urinary 8–hydroxy–2′–deoxyguanosine (8–OHdG) and genetic polymorphisms in breast cancer patients._

_Kuo HW, Chou SY, Hu TW, Wu FY, Chen DJ._

**Source**

Institute of Environmental Health, China Medical University, No. 91, Hsueh–Shin Road, Taichung, Taiwan. wukuo@mail.cmu.edu.tw

**Abstract**

Reactive oxygen species (ROS) causes damage to DNA, but the role of ROS in breast carcinoma is still not clear. The objective of this study was to measure the urinary 8–OHdG levels of breast cancer patients at each stage of carcinogenesis and assess its association with the development of breast cancer. Sixty patients with malignant breast tumors were matched with 60 control subjects of the same ages in this case control study. Urinary 8–OHdG levels were significantly higher among breast cancer patients than among the control subjects, after making adjustments for confounders such as smoking, coffee consumption and use of oral contraceptives. The breast cancer patients were divided into three groups based on the stages of their cancer; urinary 8–OHdG levels decreased with each stage of breast carcinoma. Using multiple regression and logistic models adjusted for other covariates, urinary 8–OHdG levels significantly correlated with the development of breast cancer. However, it was found that breast cancer was not significantly influenced by CYP1A1, CYP1M1 or NAT2 polymorphisms. In conclusion, it was found that oxygen radical generation occurred within carcinoma cells, but the role of polymorphism of specific genes in the development of breast cancer should be evaluated.
Oxidative damage markers as possible discriminatory biomarkers in breast carcinoma. Pande D, Negi R, Karki K, Khanna S, Khanna RS, Khanna HD.

Source
Department of Biophysics, Banaras Hindu University, Varanasi, India.

Abstract
The study was designed to evaluate the markers of oxidative damage and to establish their diagnostic utility in breast carcinoma patients. Levels of 8-hydroxy-2-deoxyguanosine (8-OH-dG), protein carbonyl (PC), and malondialdehyde (MDA) along with total antioxidant status (TAS) were measured in breast carcinoma patients and controls. Receiver operating characteristic (ROC) analysis was done to study the diagnostic potential of the oxidative damage markers. Significant increases in oxidative damage markers were observed in breast carcinoma patients compared with the normal controls, which were accompanied by significant decrease in TAS. The logistic regression analysis revealed higher levels of oxidative stress marker and reduced level of TAS were significantly associated with breast cancer. ROC curves analysis demonstrates that 8-OHdG and PC are better indicators for distinguishing cancer patients from controls, followed by MDA and TAS. Our results indicate increased oxidative damage is associated with malignancy in breast cancer patients. High accuracy of oxidative stress markers in indicating cancer presence can be used as discriminatory makers for efficient diagnosis.
**Biomarkers of breast cancer**


**Evaluation of molecular markers in a rat model of mammary carcinogenesis.**

Vinothini G, Murugan RS, Nagini S.

**Source**

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar–608 002, Tamil Nadu, India.

**Abstract**

We sought to evaluate the molecular markers involved in breast tumorigenesis in a rat model that mimics many essential elements of human breast cancer. Female Sprague–Dawley rats were divided into two groups. Animals in group 1 were given a single dose of 7,12-dimethylbenz[a]anthracene (DMBA) (20 mg/rat) dissolved in 1 ml of sesame oil by intragastric intubation. Group 2 animals received basal diet and served as control. We analyzed DMBA–induced changes in the expression of CYP isoforms (CYP1A1 and 1B1) involved in DMBA metabolism, markers of oxidative stress (4HNE, HEL, and 8–OHdG), cell survival and proliferation (PCNA, NF–kappaB–p50, NF–kappaB–p65, GST–P, and p53), apoptosis (Bcl–2, Bax, caspases, Apaf–1, cytochrome C, and Fas), invasion (uPA, MMP–2, MMP–9, TIMP–2, and RECK), and angiogenesis (VEGF, VEGF–R1, HIF–1alpha, and PLGF) by immunohistochemical localization, Western blot, and reverse transcriptase–polymerase chain reaction (RT–PCR) analysis. The present study demonstrates increased carcinogen metabolism, oxidative stress, cell proliferation, together with apoptosis evasion, invasion, metastasis, and neovascularization that may confer a selective growth advantage to DMBA–induced mammary tumors. Aberrant expression of multiple molecules in key signaling pathways in Sprague–Dawley rat mammary tumors renders this model as an important tool for monitoring carcinogenic progression and chemointervention.
Diet and biomarkers of oxidative damage in women previously treated for breast cancer.

Thomson CA, Giuliano AR, Shaw JW, Rock CL, Ritenbaugh CK, Hakim IA, Hollenbach KA, Alberts DS, Pierce JP.

Source
Department of Nutritional Sciences, The University of Arizona, Tucson 85721–0038, USA. cthomson@email.arizona.edu

Abstract
This study sought to evaluate the relationship between dietary intake of fat, polyunsaturated fat, saturated fat, arachidonic acid, and selected dietary antioxidants and levels of oxidative damage as measured by urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-epi-prostaglandin F2alpha (8-iso-PGF2alpha) in women previously treated for breast cancer. Two hundred two study subjects participating in the Women's Healthy Eating and Living (WHEL) study were included in this ancillary study. Dietary intakes and concentrations of urinary 8-OHdG and 8-iso-PGF2alpha were measured at baseline and 12 mo in the 179 women included in the analytical cohort. Study subjects demonstrated a significant reduction in dietary total, polyunsaturated, and saturated fat intake and a significant increase in vitamins E and C and beta-carotene intake from baseline to 12 mo. Linear mixed–models analysis using baseline and Year 1 data indicated that vitamin E intake was inversely associated with both 8-OHdG and 8-iso-PGF2alpha. 8-Iso-PGF2alpha is increased with increased body mass index (BMI) and polyunsaturated fatty acid (PUFA) intake, indicating an increase in lipid peroxidation with greater BMI and higher PUFA intake. 8-OHdG was inversely related to age but positively related to arachidonic acid, indicating an increase in DNA damage with higher intake of arachidonic acid (meat). The results of this nested case–controlled study provide potential mechanisms by which a high fruit and vegetable, low–fat diet might reduce the recurrence rate of or early–stage breast cancer.
8–OHdG and Tea


Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols.

Weisburger JH, Chung FL.

Source
American Health Foundation, One Dana Road, Valhalla, NY 10595, USA. jweisbur@ahf.org

Abstract
The beverage tea, from the top leaves of the plant Camellia sinensis is one of the most widely used beverages in the world, second only to water. Black and green tea have mostly similar actions. The active components are polyphenols, mainly epigallocatechin gallate in green tea, and the tea leaf polyphenol oxidase mediated oxidation to oolong and black tea, yielding other polyphenols, theaflavin and thearubigins. There is 40–50 mg caffeine in a 160–ml cup of tea. The chemopreventive effects of tea depend on: (1) its action as an antioxidant; (2) the specific induction of detoxifying enzymes; (3) its molecular regulatory functions on cellular growth, development and apoptosis; and (4) a selective improvement in the function of the intestinal bacterial flora. The oxidation of LDL cholesterol, associated with a risk for atherosclerosis and heart disease, is inhibited by tea. Many of cancers are caused by lifestyle elements. One is cigarette and tobacco use, leading to cancer in the oral cavity, esophagus and lung, inhibited by tea. Mice administered a tobacco nitrosamine, 4–(methylnitrosamino)–1–(3–pyridyl)–1–butanone (NNK), developed significantly fewer lung tumors than controls when given green tea or its major polyphenol, epigallocatechin gallate (EGCG). Tea suppressed the formation of 8–hydroxydeoxyguanosine (8–OHdG), a marker of oxidative DNA damage, in the lung DNA of mice given NNK. Gastric cancer, caused by a combination of Helicobacter pylori and salted foods, is lower in tea drinkers. Western nutritionally–linked cancers of the breast, colon, prostate and pancreas can be inhibited by tea. The formation of genotoxic carcinogens for these target organs during the cooking of meats, heterocyclic amines, and their effects were decreased by tea. Tea inhibited the formation of reactive oxygen species and radicals and induced cytochromes P450 1A1, 1A2 and 2B1, and glucuronosyl transferase. The higher formation of glucuronides represents an important mechanism in detoxification. The developmental aspects and growth of cancers through promotion are decreased by tea. The regular use of a widely available, tasty, inexpensive beverage, tea, has displayed valuable preventive properties in chronic human diseases.
Upregulation of Nrf2 is protective from breast cancer and 8-OHdG helps to monitor this


Induction of NAD(P)H–quinone oxidoreductase 1 by antioxidants in female ACI rats is associated with decrease in oxidative DNA damage and inhibition of estrogen–induced breast cancer.

Singh B, Bhat NK, Bhat HK.

Source
Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri–Kansas City, Kansas City, MO 64108, USA.

Abstract
Exact mechanisms underlying the initiation and progression of estrogen–related cancers are not clear. Literature, evidence and our studies strongly support the role of estrogen metabolism–mediated oxidative stress in estrogen–induced breast carcinogenesis. We have recently demonstrated that antioxidants vitamin C and butylated hydroxyanisole (BHA) or estrogen metabolism inhibitor α–naphthoflavone (ANF) inhibit 17β–estradiol (E2)–induced mammary tumorigenesis in female ACI rats. The objective of the current study was to identify the mechanism of antioxidant–mediated protection against E2–induced DNA damage and mammary tumorigenesis. Female ACI rats were treated with E2 in the presence or absence of vitamin C or BHA or ANF for up to 240 days. Nuclear factor erythroid 2–related factor 2 (NRF2) and NAD(P)H–quinone oxidoreductase 1 (NQO1) were suppressed in E2–exposed mammary tissue and in mammary tumors after treatment of rats with E2 for 240 days. This suppression was overcome by co–treatment of rats with E2 and vitamin C or BHA. Time course studies indicate that NQO1 levels tend to increase after 4 months of E2 treatment but decrease on chronic exposure to E2 for 8 months. Vitamin C and BHA significantly increased NQO1 levels after 120 days. 8–Hydroxydeoxyguanosine (8–OHdG) levels were higher in E2–exposed mammary tissue and in mammary tumors compared with age–matched controls. Vitamin C or BHA treatment significantly decreased E2–mediated increase in 8–OHdG levels in the mammary tissue. In vitro studies using silencer RNA confirmed the role of NQO1 in prevention of oxidative DNA damage. Our studies further demonstrate that NQO1 upregulation by antioxidants is mediated through NRF2.
Nrf2: Master Regulator of Cytoprotection

- Enhances Cytoprotection
- Increases Detoxification
- Regulates production of Phase 2 Enzymes
- Enhances stability and turnover of proteins
- Reduces inflammation
- Protects against neurodegeneration
- Anti-tumorigenic
- Promotes longevity
Treatment options

- Curcumin
- Green Tea
- Sulforaphane from broccoli
- Resveratrol
- Oxidative therapies

Reduced formation of depurinating estrogen–DNA adducts by sulforaphane or KEAP1 disruption in human mammary epithelial MCF–10A cells.

Yang L1, Zahid M, Liao Y, Rogan EG, Cavalieri EL, Davidson NE, Yager JD, Visvanathan K, Groopman JD, Kensler TW.

Author information

Abstract

Sulforaphane (SFN) is a potent inducer of detoxication enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione–S–transferase (GST) via the Kelch–like erythroid–derived protein with CNC homology–associated protein 1 (Keap1)–NF–E2–related factor 2 (Nrf2) signaling pathway. NQO1 reduces the carcinogenic estrogen metabolite, catechol estrogen–3,4–quinone, whereas GSTs detoxify it through conjugation with glutathione. These 3,4–quinones can react with DNA to form depurinating DNA adducts. Thus, SFN may alter estrogen metabolism and thus protect against estrogen–mediated DNA damage and carcinogenesis. Human breast epithelial MCF–10A cells were treated with either vehicle or SFN and either estradiol (E2) or its metabolite 4–hydroxyestradiol (4–OHE2). 4–Hydroxy–derived estrogen metabolites and depurinating DNA adducts formed from E2 and its interconvertable metabolite estrone (E1) were analyzed by mass spectrometry. Levels of the depurinated adducts, 4–OHE1/2–1–N3Adenine and 4–OHE1/2–1–N7Guanine, were reduced by 60% in SFN–treated cells, whereas levels of 4–OCH3E1/2 and 4–OHE1/2–glutathione conjugates increased. To constitutively enhance the expression of Nrf2–regulated genes, cells were treated with either scrambled or siKEAP1 RNA. Following E2 or 4–OHE2 treatments, levels of the adenine and guanine adducts dropped 60–70% in siKEAP1–treated cells, whereas 4–OHE1/2–glutathione conjugates increased. However, 4–OCH3E1/2 decreased 50% after siKEAP1 treatment. Thus, treatment with SFN or siKEAP1 has similar effects on reduction of depurinating estrogen–DNA adduct levels following estrogen challenge. However, these pharmacologic and genetic approaches have different effects on estrogen metabolism to O–methyl and glutathione conjugates. Activation of the Nrf2 pathway, especially elevated NQO1, may account for some but not all of the protective effects of SFN against estrogen–mediated DNA damage.
Regulation of Mitochondrial Biogenesis by PGC-1α and Nrf2

Cold, fasting

Exercise

Retrograde signaling?

Nitric oxide

cGMP

CREB

MEF-2

NRF-1

NRF-2

ERRα

PGC-1α

CaMKIV

CalcineurinA

cAMP

Ca++

Muscle-specific COX subunits

Import/assembly

Translation

Respiratory chain

mtDNA transcription/replication

Fatty acid oxidation

Energy deprivation (exercise)
**Inhibition of estrogen signaling activates the NRF2 pathway in breast cancer.**

**Yao Y, Brodie AM, Davidson NE, Kensler TW, Zhou Q.**

**Source**
Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

**Abstract**
Exposure to higher levels of estrogen produces genotoxic metabolites that can stimulate mammary tumorigenesis. Induction of NF-E2–related factor 2 (NRF2)–dependent detoxifying enzymes (e.g., NAD(P)H–quinone oxidoreductase 1 (NQO1)) is considered an important mechanism of protection against estrogen–associated carcinogenesis because they would facilitate removal of toxic estrogens. Here, we studied the impact of estrogen–receptor (ER) signaling on NRF2–dependent gene transcription. In luciferase assay experiments using the 5′-flanking region of the human NQO1 gene promoter, we observe that ERα ligand–binding domain (LBD) is required for estrogen inhibition of NQO1 promoter activity in estrogen–dependent breast cancer cells. Chromatin immunoprecipitation (ChIP) assay shows that estrogen recruits ERα and a class III histone deacetylase SIRT1 at the NQO1 promoter, leading to inhibition of NQO1 transcription. Inhibition of ERα expression by the antiestrogen shikonin reverses the inhibitory effect of estrogen on NQO1 expression. As a consequence, a chemoprevention study was undertaken to monitor the impact of shikonin on DNA lesions and tumor growth. **Treatment of MCF–7 breast cancer cells with shikonin inhibits estrogen–induced 8–hydroxy–2–deoxyguanosine (8–OHdG), a marker of DNA damage.** NQO1 deficiency promotes estrogen–dependent tumor formation, and shikonin inhibits estrogen–dependent tumor growth in an NQO1–dependent manner in MCF–7 xenografts. These results suggest that estrogen–receptor signaling pathway has an inhibitory effect on NRF2–dependent enzymes. Moreover, shikonin reverses the inhibitory effects of estrogen on this pathway and may contribute to breast cancer prevention.
Antioxidant Status in Breast Cancer


DNA oxidation and antioxidant status in breast cancer.
Himmetoglu S, Dincer Y, Ersoy YE, Bayraktar B, Celik V, Akcay T.

Source
Department of Biochemistry, and Department of General Surgery, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey.

Abstract

PURPOSE: Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer. The objective of this study was to determine 8-hydroxydeoxyguanosine (8-OHdG) level as a marker of oxidative DNA damage, glutathione peroxidase (G-Px), and superoxide dismutase (SOD) activities as antioxidant activity, in sera from women with breast cancer.

METHODS: Forty-nine patients with malign breast tumor were included in the study. Blood samples were collected before the surgical operation. Serum level of 8-OHdG was measured with a competitive enzyme-linked immunosorbent assay kit, SOD, and G-Px activities were measured by spectrophotometric kits.

RESULTS: 8-Hydroxydeoxyguanosine level and SOD activity were found to be increased in breast cancer group as compared with control group. Glutathione peroxidase activity in the breast cancer group was lower than those in the control group. The ratio of 8-OHdG/G-Px in breast cancer patients was found to be higher than those in the controls. There were correlations between 8-OHdG and CA19–9 (r = 0.77; P < 0.01); age and G-Px (r = –0.84; P < 0.05) in the breast cancer group.

CONCLUSIONS: Data show that serum levels of 8-OHdG and SOD activities are higher in patients with breast cancer. Glutathione peroxidase activity is lower in the breast cancer group. Increased ratio of 8-OHdG/G-Px in breast cancer patients is the evidence for impaired oxidant/antioxidant balance in breast cancer.
Oxidative Stress and Tamoxifen Therapy


Singh B, Bhat NK, Bhat HK.

Source
Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri–Kansas City, Kansas City, Missouri, United States of America.

Abstract
Epidemiological and experimental evidences strongly support the role of estrogens in breast tumor development. Both estrogen receptor (ER)–dependent and ER–independent mechanisms are implicated in estrogen–induced breast carcinogenesis. Tamoxifen, a selective estrogen receptor modulator is widely used as chemoprotectant in human breast cancer. It binds to ERs and interferes with normal binding of estrogen to ERs. In the present study, we examined the effect of long–term tamoxifen treatment in the prevention of estrogen–induced breast cancer. Female ACI rats were treated with 17β–estradiol (E2), tamoxifen or with a combination of E2 and tamoxifen for eight months. Tissue levels of oxidative stress markers 8–iso–Prostane F(2α) (8–isoPGF(2α)), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and oxidative DNA damage marker 8–hydroxydeoxyguanosine (8–OHdG) were quantified in the mammary tissues of all the treatment groups and compared with age–matched controls. Levels of tamoxifen metabolizing enzymes cytochrome P450s as well as estrogen responsive genes were also quantified. At necropsy, breast tumors were detected in 44% of rats co–treated with tamoxifen+E2. No tumors were detected in the sham or tamoxifen only treatment groups whereas in the E2 only treatment group, the tumor incidence was 82%. Co–treatment with tamoxifen decreased GPx and catalase levels; did not completely inhibit E2–mediated oxidative DNA damage and estrogen–responsive genes monoamine oxygenase B1 (MaoB1) and cell death inducing DFF45 like effector C (Cidec) but differentially affected the levels of tamoxifen metabolizing enzymes. In summary, our studies suggest that although tamoxifen treatment inhibits estrogen–induced breast tumor development and increases the latency of tumor development, it does not completely abrogate breast tumor development in a rat model of estrogen–induced breast cancer. The inability of tamoxifen to completely inhibit E2–induced breast carcinogenesis may be because of increased estrogen–mediated oxidant burden.
Neurologic Implications
When initiated soon after menopause, hormone therapy with estradiol prevented degeneration in key brain regions of women who were at heightened dementia risk, according to a new study led by Stanford University School of Medicine researchers.

The investigators also found that another type of hormone therapy, marketed under the brand name Premarin, was far less protective. Other Premarin components exert various endocrinological effects on different tissues.
The psychostimulants d-amphetamine and methylphenidate are thought to be the most effective treatment in children, adolescents, and adults with attention deficit–hyperactivity disorder (ADHD) because they potentiate both dopamine (DA) and norepinephrine (NE) at the synaptic cleft. These medications are not free from side effects and controversy. Newer effective and safe treatments are needed. S–Adenosyl–L–methionine (SAM), the active form of methionine, acts as a methyl donor and is involved in many metabolic pathways. It has beta adrenergic and DA receptor agonist activity. We have been using oral SAM in a sample of well–diagnosed adults with ADHD, residual state (RS) in a 4–week open trial to establish SAM effectiveness and safety and in a 9–week, double–blind, placebo–controlled crossover trial. Preliminary data from the open trial reveal that 75 percent (6 out of 8 male) patients improve on it. The 2 who did not improve had not improved on methylphenidate trial. Improvement ranged from moderate to marked, with minimal and transient side effects that did not interfere with functioning.
Estrogen and Dopamine

- Neuroscience & Biobehavioral Reviews
- Volume 24, Issue 1, January 2000, Pages 143–147
- Neuroprotective effects of estradiol in mesencephalic dopaminergic neurons
- ---women have less ADHD, onset increases postmenopausally, women have less Parkinson’s
Oxidative stress, aberrant methylation and Neurologic conditions

The role for oxidative stress in aberrant DNA methylation in Alzheimer's disease.
Fleming JL¹, Phiel CJ, Toland AE.
Author information
Abstract
Alzheimer's disease (AD) is a common, progressive neurodegenerative disorder without highly effective therapies. The etiology of AD is heterogeneous with amyloid–beta plaques, neurofibrillary tangles, oxidative stress, and aberrant DNA methylation all implicated in the disease pathogenesis. DNA methylation is a well-established process for regulating gene expression and has been found to regulate a growing number of important genes involved in AD development and progression. Additionally, aberrations in one–carbon metabolism are a common finding in AD patients with individuals exhibiting low S–adenosylmethionine and high homocysteine levels as well as low folate and vitamin B. Oxidative stress is considered one of the earliest events in AD pathogenesis and is thought to contribute largely to neuronal cell death. Emerging evidence suggests an interaction exists between oxidative stress and DNA methylation; however, the mechanism(s) remain unclear. This review summarizes known and potential genes implicated in AD that are regulated by DNA methylation and oxidative stress. We also highlight the evidence for the role of oxidative damage contributing to DNA hypomethylation in AD patients through several mechanisms as well as implications for disease understanding and therapeutic development.
Alzheimer’s and “Estrogen”

In an ancillary study of the Women’s Health Initiative involving relatively healthy postmenopausal women aged 65 to 79 years, conjugated estrogens combined with a progestin (medroxyprogesterone acetate) compared to placebo doubled the risk of dementia.\textsuperscript{72}

This risk represented about 2.3 additional cases of dementia per year, per 1000 women in the age group studied. Among women without a uterus, conjugated estrogens without medroxyprogesterone acetate did not affect dementia risk ($p = 0.2$), although risk was significantly increased in the combined analysis of women with and without a uterus.\textsuperscript{73}

\textit{Climacteric}. 2014 Dec; 17(0 2): 38–46.
Published online 2014 Aug 17. doi: 10.3109/13697137.2014.929650
Three Midlife Strategies to Prevent Cognitive Impairment Due to Alzheimer’s Disease
Walk For The Brain

- Exercise might also reduce Alzheimer risk indirectly through threshold modification. This may involve effects on inflammation, oxidative stress, and immune modulation. In both animals and humans, aerobic exercise increases levels of brain derived neurotrophic factor and other growth factors.
- Higher serum levels of brain derived neurotrophic factor are linked to a lower risk of developing Alzheimer’s disease.
- These proteins support neuronal survival, enhance synaptic plasticity, promote new blood vessel formation, and lead to the formation of new neurons in the dentate gyrus of the hippocampus.
- There is indirect evidence that exercise induces neurogenesis in the human dentate gyrus.
- In a clinical trial involving 120 healthy older adults, aerobic walking over a one year period of time, compared to a non-aerobic control intervention, increased the volume of the hippocampus measured by magnetic resonance imaging; this temporal lobe region is critically involved in memory encoding and consolidation. Interestingly, brain derived neurotrophic factor might also mediate cognitive reserve linked to mental activity.

Three Midlife Strategies to Prevent Cognitive Impairment Due to Alzheimer’s Disease
Immune Balancing
Autoimmune Conditions Flared by Oxidative Stress which influences Demethylation

Oxidative Stress, T Cell DNA Methylation and Lupus.
Li Y, Gorelik G, Strickland FM, Richardson BC.

Abstract
Objective: Lupus develops when genetically predisposed people encounter environmental agents such as UV light, silica, infections and cigarette smoke that cause oxidative stress, but how oxidative damage modifies the immune system to cause lupus flares is unknown. We reported that inhibiting DNA methylation in CD4+ T cells by blocking ERK pathway signaling is sufficient to alter gene expression, and that the modified cells cause lupus-like autoimmunity in mice.
We also reported that T cells from patients with active lupus have decreased ERK pathway signaling, decreased DNA methylation, and overexpress genes normally suppressed by DNA methylation. We therefore tested whether oxidizing agents decrease T cell ERK pathway signaling, decrease DNA methyltransferase levels, and cause demethylation and overexpression of T cell genes similar to that found in T cells from patients with active lupus. Methods: CD4+ T cells were treated with the oxidizers H2O2 or ONOO-. Effects on ERK pathway signaling were measured by immunoblotting, Dnmt1 levels by RT-PCR, and methylation and expression of T cell genes were measured using flow cytometry, RT-PCR and bisulfite sequencing. Results: H2O2 and ONOO- inhibited T cell ERK pathway signaling by inhibiting the upstream regulator PKCδ, decreased Dnmt1 levels, and caused demethylation and overexpression genes previously shown to be suppressed by DNA methylation in T cells from patients with active lupus. Conclusions: Oxidative stress may contribute to human lupus flares by inhibiting T cell ERK pathway signaling to decrease Dnmt1 and cause DNA demethylation. © 2014 American College of Rheumatology.
Oxidative damage and transmethylation micronutrient effects on the T cell epigenome in lupus (P5141)

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The development of human lupus is attributed in part to T cell DNA demethylation. The mechanisms causing T cell DNA demethylation in lupus patients are poorly understood. DNA methylation is dependent on the methyl donor S-adenosylmethionine (SAM) and DNA methyltransferase (Dnmt) activity, and is inhibited by S-adenosylhomocysteine (SAH). Several dietary nutrients affect SAM levels including folate and methionine. Additionally, Dnmt levels are low in lupus patients due to defective ERK pathway signaling, caused by oxidative stress resulting in PKC nitration. We hypothesized that T cells subjected to oxidative stress will overexpress genes normally suppressed by DNA methylation due to low Dnmt levels, and the overexpression will increase in the presence of low folate and methionine levels. We treated PHA-stimulated human T cells with the oxidant peroxynitrite, cultured the cells in media containing high or low folate or methionine levels, and compared the effects on expression of the methylation sensitive genes CD70 and KIR. Peroxynitrite treated T cells cultured in lower nutrient concentrations overexpressed CD70 and KIR compared to those cultured in higher concentrations and/or no oxidant. These results suggest that low nutrient levels may increase T cell epigenetic changes caused by oxidation. Since several environmental insults resulting in oxidative stress are associated with lupus, dietary supplementation may mitigate the damage.
MS and Estriol

- Medscape Medical News > Conference News
- Adding Estriol Reduces MS Relapse Rate
- Pauline Anderson
- April 30, 2014
- --47% reduction in episodes of MS at year one and 35% by year two.

9. Gastric secretion, proinflammatory cytokines and epidermal growth factor (EGF) in the delayed healing of lingual and gastric ulcerations by testosterone. Inflammapharmacology; 2008 Feb; 16(1):40–7

Estradiol decreases colonic permeability through estrogen receptor beta-mediated up-regulation of occluding and junctional adhesion molecule–A in epithelial cells.

Leaky gut and autoimmune diseases. Mucosal Biology Research Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA. afasano@mbrc.umaryland.ed

Autoimmune diseases are characterized by tissue damage and loss of function due to an immune response that is directed against specific organs. This review is focused on the role of impaired intestinal barrier function on autoimmune pathogenesis. Together with the gut–associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to non–self antigens. Zonulin is the only physiologic modulator of intercellular tight junctions described so far that is involved in trafficking of macromolecules and, therefore, in tolerance/immune response balance. When the zonulin pathway is deregulated in genetically susceptible individuals, autoimmune disorders can occur. This new paradigm subverts traditional theories underlying the development of these diseases and suggests that these processes can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing the zonulin–dependent intestinal barrier function. Both animal models and recent clinical evidence support this new paradigm and provide the rationale for innovative approaches to prevent and treat autoimmune diseases.
Estradiol helped to maintain a certain microbiome

E2 group maintained a normal level of Firmicutes to Bacteroides

By maintaining normal flora weight gain was prevented
Conclusions

Intestinal microbial richness and functions, including but not limited to \( \beta \)-glucuronidase, influence levels of non-ovarian estrogens via enterohepatic circulation. Thus, the gut microbial community may contribute to the risk for estrogen–related conditions in older adults. Understanding how *Clostridia* taxa relate to systemic estrogens may identify targets for interventions.
Estrogen metabolism requires a functional estrobolome

Gut microbial functions driving estrogen metabolism and contributing to the proportions of recirculated and excreted estrogens and estrogen metabolites has long been considered (Adlercreutz and Jarvenpaa, 1982; Eriksson, 1970).
The final piece of elimination occurs in the gut. After conjugation of hormones, they are water soluble and are prepared to be excreted through the feces. If bowel function is impaired, hormones can sit in the gut longer, and be reabsorbed.

Also in a state of dysbiosis the enzyme Beta–glucoronidase may be affected. This relationship is demonstrated by the production of daidzein from soy and other herbs which is created by human intestinal bacteria.

This metabolite, from beneficial gut flora and soy, potently inhibits beta–glucuronidase. [29]

Beta–glucuronidase will cleave hormones from their conjugates resulting in an increase of reabsorption. Direct evidence of the relationship of gut flora to hormones is seen with research that associates antibiotic use with breast cancer.

It also changes the way phytochemicals such as phytoestrogens and polyphenols are metabolized. This dysbiosis results in a loss of formation of compounds that are protective against breast cancer. [30, 31]
Treatment of Gut/Hormone

- Calcium and Potassium D–glucarate inhibit beta–glucuronidase
- Probiotics
Oxidative Stress; The Methylation Master
Figure 1. Redox and methylation–related pathways in neurons.


http://www.plosone.org/article/info:doi/10.1371/journal.pone.0056927
Healthy Mitochondria dictate methylation

- Healthy Mitochondria make ATP
- ATP + Methionine → SAMe
- Mitochondria decrease Reactive Oxygen Species
- Mitochondria main producer of Reactive Oxygen Species
- Estrogen Drives Mitochondrial health
- Methylation Caution: Tissue specific, pH specific, enzyme/protein/DNA specific
Taurine, glutathione and bioenergetics.
Hansen SH, Grunnet N.

Source
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Abstract
Biochemistry textbook presentations of bioenergetics and mitochondrial function normally focus on the chemiosmotic theory with introduction of the tricarboxylic acid cycle and the electron transport chain, the proton and electrical gradients and subsequent oxidative phosphorylation and ATP–production by ATP synthase. The compound glutathione (GSH) is often mentioned in relation to mitochondrial function, primarily for a role as redox scavenger. Here we argue that its role as redox pair with oxidised glutathione (GSSG) is pivotal with regard to controlling the electrical or redox gradient across the mitochondrial inner–membrane. The very high concentration of taurine in oxidative tissue has recently led to discussions on the role of taurine in the mitochondria, e.g. with taurine acting as a pH buffer in the mitochondrial matrix. A very important consequence of the slightly alkaline pH is the fact that the NADH/NAD(+) redox pair can be brought into redox equilibrium with the GSH redox pair GSH/GSSG. An additional consequence of having GSH as redox buffer is the fact that from the pH dependence of its redox potential, it becomes possible to explain that the mitochondrial membrane potential has been observed to be independent of the matrix pH. Finally a simplified model for mitochondrial oxidation is presented with introduction of GSH as redox buffer to stabilise the electrical gradient, and taurine as pH buffer stabilising the pH gradient, but simultaneously establishing the equilibrium between the NADH/NAD(+) redox pair and the redox buffer pair GSH/GSSG.
Glutathione is critical to mitochondrial function

Glutathione and γ-glutamylcysteine in the antioxidant and survival functions of mitochondria.
Quintana–Cabrera R, Bolaños JP.

Source
Institute for Functional Biology and Genomics (IBFG), Department of Biochemistry and Molecular Biology, University of Salamanca–CSIC, Zacarias Gonzalez 2, 37007 Salamanca, Spain.

Abstract
Mitochondria are both the main producers and targets of ROS (reactive oxygen species). Among the battery of antioxidants that protect mitochondria from ROS, GSH is thought to be essential for the organelle antioxidant function. However, mitochondria cannot synthesize GSH de novo, thus depending on an efficient transport from the cytosol to maintain their redox status. In the present article, we review recent data suggesting that the cellular redox control might not be the main function of GSH, and that its immediate precursor, γGC (γ-glutamylcysteine), can take over the antioxidant role of GSH and protect the mitochondria from excess ROS. Together, GSH and γGC may thus represent an as yet unrecognized defence system relevant for degenerative processes associated with the imbalance in the cellular redox control.
Low glutathione causes hypomethylation


Blood glutathione redox status and global methylation of peripheral blood mononuclear cell DNA in Bangladeshi adults.


Author information

Abstract

Oxidative stress and DNA methylation are metabolically linked through the relationship between one-carbon metabolism and the transsulfuration pathway, but possible modulating effects of oxidative stress on DNA methylation have not been extensively studied in humans. Enzymes involved in DNA methylation, including DNA methyltransferases and histone deacetylases, may show altered activity under oxidized cellular conditions. Additionally, in vitro studies suggest that glutathione (GSH) depletion leads to global DNA hypomethylation, possibly through the depletion of S-adenosylmethionine (SAM). We tested the hypothesis that a more oxidized blood GSH redox status is associated with decreased global peripheral blood mononuclear cell (PBMC) DNA methylation in a sample of Bangladeshi adults. Global PBMC DNA methylation and whole blood GSH, glutathione disulfide (GSSG), and SAM concentrations were measured in 320 adults. DNA methylation was measured by using the [(3)H]-methyl incorporation assay; values are inversely related to global DNA methylation. Whole blood GSH redox status (Eh) was calculated using the Nernst equation. We found that a more oxidized blood GSH Eh was associated with decreased global DNA methylation (B ± SE, 271 ± 103, p = 0.009). Blood SAM and blood GSH were associated with global DNA methylation, but these relationships did not achieve statistical significance. Our findings support the hypothesis that a more oxidized blood GSH redox status is associated with decreased global methylation of PBMC DNA. Furthermore, blood SAM does not appear to mediate this association. Future research should explore mechanisms through which cellular redox might influence global DNA methylation.
High 8–ohdg associated methylation defects in cancer/tumor suppressor genes


Reactive oxygen species induce epigenetic instability through the formation of 8–hydroxydeoxyguanosine in human hepatocarcinogenesis.

Nishida N¹, Arizumi T, Takita M, Kitai S, Yada N, Hagiwara S, Inoue T, Minami Y, Ueshima K, Sakurai T, Kudo M.

Author information

Abstract

Chronic hepatitis C (CHC) triggers oxidative stress and contributes to the emergence of hepatocellular carcinoma (HCC). We previously reported that tumor suppressor gene (TSG) methylation is a critical factor during the early stages of hepatocarcinogenesis. In this study, we clarify the association between oxidative stress and epigenetic alterations during hepatocarcinogenesis. We examined DNA oxidation and methylation profiles in 128 liver biopsy samples from CHC patients. The DNA oxidation and methylated TSG numbers were quantified using immunohistochemical analysis of 8–hydroxydeoxyguanosine (8–OHdG) and quantitative PCR for 11 TSGs, respectively. The quantitative chromatin immunoprecipitation–PCR (ChIP–qPCR) assay in HepG2 and fetal liver Hc cells treated with H2O2 was used to quantify trimethyl–H3K4, acetylated–H4K16 (an active chromatin marker), trimethyl–H3K27 (a repressive chromatin marker) and 8–OHdG. We analyzed 30 promoters of 25 different TSGs by qPCR. The high levels of 8–OHdG was the only variable that was significantly associated with the increased number of methylated TSGs in CHC (p < 0.0001). The ChIP–qPCR revealed that after H2O2 treatment of the cell lines, the 8–OHdG–bound promoters showed a modification from an active chromatin (trimethyl–H3K4 and acetylated–H4K16 dominant) to a repressive chromatin (trimethyl–H3K27 dominant) status. We conclude that oxidative stress alters the chromatin status, which leads to abnormal methylation of TSGs, and contributes to hepatocarcinogenesis in CHC patients.
Methionine Synthase

Inhibited by:
- Toxins
- Lead
- Mercury
- Oxidative Stress
Amino Acids and Hormones

- Amino acids status is important to consider alongside hormone therapy. One must always keep in mind the question of what the primary function of hormones is. In the case of estrogen its most basic biological function is to prepare the uterine lining to sustain an embryo. The follicular phase must facilitate rapid growth of tissue. Amino acid substrate must be plentiful for this function to occur.

- Estrogen also has anabolic effects elsewhere in the body, which are impossible to perform unless nutrition is adequate. In fact as estradiol increases, the activity of branched chain alpha-dehydrogenase complex is decreased to increase the amount of essential amino acids available.

- Variations in break-down of branched chain amino acids are seen in females and not males that correlate with levels of estrogen. [32] It can be postulated that the full effects of estrogen cannot be achieved without adequate amino acids present.
Liver clearance

- All metabolites are conjugated by the liver either through glucuronidation or sulfation. This illustrates how important liver metabolism and more fundamentally adequate nutritional status is to hormone regulation.

- Hormones will not be properly regulated if detoxification pathways are not adequately functioning.

- Low amino acid status will alter conjugation.

- S–adenosyl–methionine is necessary to prepare metabolites for conjugation, and Glutathione as well as sulfur containing amino acids are required for elimination.
Transdermal doesn’t increase CRP

**Abstract**

OBJECTIVES: We investigated whether the route of estrogen replacement therapy (ET) is the major determinant of C-reactive protein (CRP) in postmenopausal women.

BACKGROUND: Recent studies demonstrated that oral ET causes a sustained increase in CRP, implicating a proinflammatory effect. Because CRP is synthesized in the liver, we hypothesized that estrogen-induced CRP elevation is related to first-pass hepatic metabolism.

METHODS: In 21 postmenopausal women, we conducted a randomized, crossover, placebo–controlled study to compare the effects of transdermal versus oral ET on CRP and inflammatory cytokines. We measured CRP, interleukin (IL)–1β, IL–6, and tumor necrosis factor–alpha before and after eight weeks of transdermal estradiol (E(2)) (100 microg/day), oral conjugated estrogen (CEE) (0.625 mg/day), or placebo. Insulin–like growth factor–1 (IGF–1), a hepatic–derived anabolic peptide, was also measured.

RESULTS: Transdermal E(2) had no effect on CRP or IGF–1 levels. In contrast, eight weeks of oral conjugated estrogens caused a more than twofold increase in CRP and a significant reduction in IGF–1 (p < 0.01) in the same women. The magnitude of increase in CRP was inversely correlated to the decrease in IGF–1 (r = −0.49, p = 0.008). Neither transdermal E(2) nor oral CEE had any effects on the plasma concentrations of cytokines that promote CRP synthesis.

CONCLUSIONS: In postmenopausal women, oral but not transdermal ET increased CRP by a first-pass hepatic effect. An increase in CRP levels is accompanied by a reduction in IGF–1, an anti–inflammatory growth factor. Because CRP is a powerful predictor of an adverse prognosis in otherwise healthy postmenopausal women, the route of administration may be an important consideration in minimizing the adverse effects of ET on cardiovascular outcomes.
Involvement of Oxidative Stress in Age–Related Bone Loss.
Zhang YB, Zhong ZM, Hou G, Jiang H, Chen JT.

Department of Orthopedic and Spinal Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China.

BACKGROUND:
Age–related bone loss is a primary factor in osteoporosis and osteoporotic fractures in the elderly. Although oxidative stress was reported to play an important role in aging and postmenopausal bone loss, data on relating oxidative stress to age–related bone loss were scanty. This study aimed to investigate whether oxidative stress is involved in age–related bone loss.

MATERIALS AND METHODS:
Young, adult, and old male Wistar rats were used in this study. Each group consisted of 26 animals. Oxidative stress parameters, such as advanced oxidation protein products (AOPP), malondialdehyde (MDA), and superoxide dismutase (SOD), were measured in the plasma and right femur homogenates. Bone mineral density (BMD) of left femurs and histomorphometry of tibias were investigated.

RESULTS:
In the plasma and femurs, the levels of AOPP and MDA were increased and the SOD activity was decreased with aging. Femur BMD decreased significantly in old rats. Bone histomorphometry indicated decreases in cancellous bone volume, trabecular thickness, percent labeled perimeter, mineral apposition rate, and bone formation rate with aging. The AOPP levels in plasma and femur, and MDA levels in the plasma were negatively correlated with the femur BMD. The SOD activity in plasma and femur was positively correlated with the femur BMD.

CONCLUSIONS:
Increase of oxidative stress and bone loss appear with aging. Oxidative stress is involved in age–related bone loss and might play an important role in the pathology of age–related bone loss.
Leptin flips the estrogen cancer switch to on (Nature, 2012)

- Adipokines such as leptin are emerging as key signaling molecules linking obesity and metabolic imbalances to inflammatory-immune diseases such as cancer and autoimmunity.
- Elevated leptin levels increase the "pro-growth" estrogen pool by enhancing genetic expression of CYP19A1 (aromatase) in estrogen-responsive tissue, thereby increasing the aromatization of androgens to estradiol (E2).
- Leptin activates the transcription factor of estrogen receptor-alpha.
- Leptin acts as a "chemoattractant" through inducing macrophage-mediated production of inflammatory mediators; including but not limited to leukotriene B4, TNF, IL-1 and IL-6.
- Leptin down regulates regulatory T cells (Treg) leading to impaired peripheral immune tolerance and low-grade chronic inflammation.
- Leptin stimulates VEGF, increasing angiogenesis.
Antioxidant Glutathione also responsible to detox the hormones

- Glutathione S transferase also appears to be upregulated by the sulfur constituents in cruciferous vegetables Brassica vegetables also improve glucuronidation aiding with elimination of estrogen metabolites
A firm link between female reproductive history and increased risk of developing cancer in the breast and endometrium has been established from epidemiological studies (1–4).

The longer women are exposed to estrogens, either through early menarche and late menopause and/or through estrogen replacement therapy, the higher is the risk of developing certain hormone-dependent cancers.

It used to be thought that the purported benefits of estrogen replacement therapy, which included the relief of menopausal symptoms, decrease in coronary heart disease, osteoporosis, stroke, and Alzheimer’s disease, justified the use of long-term estrogen replacement therapy. However, the release of the initial results from the Women’s Health Initiative Study in July 2002 cast serious doubt on this paradigm for the treatment of post-menopausal women (5).

The estrogen plus progestin arm was halted three years early due to significant increases in breast cancer, coronary heart disease, stroke, and pulmonary embolism, with more recent data suggesting an increase in vascular dementia in women over 65 on estrogen replacement therapy (6). In 2004, the estrogen arm was halted because of increased incidence of stroke (7).

A recent analysis of data from the National Cancer Institute’s Surveillance, Epidemiology, and End Results (SEER) registries showed that age-adjusted incidence rate of breast cancer fell sharply (6.7%) in 2003 compared to 2002, which seemed to be related to the drop in the use of HRT (8).

Finally, a reanalysis of nine prospective studies has shown that exposure to estrogens is associated with an increase in breast cancer risk with evidence of a dose-response relationship (9). These troubling findings highlight the urgent need for a full understanding of all the deleterious effects of estrogens including their potential to initiate and/or promote the carcinogenic process.
References

(9). Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: rea
Are Hormones the Only Answer?

- Nutrient levels (to reduce risk of heart disease, bone density, etc.)
- Adrenal Function (to make reproductive precursors)
- Liver function/detox (to keep levels stable in the body)
- Gut function (to eliminate and process appropriately)
- Reduce The Oxidative Load