Hormones, Organic Acids and Genetics: Clinical Synergy for Integrative Medicine

Mark Newman, MS
Financial Disclosure: President, Precision Analytical Lab

Non-CME/ 1 CE
Standard Testing: CBC, CMP, Lipids, etc.

Basic Hormones
(Thyroid, Sex, Adrenal, Vit D)

G.I., Advanced Hormones

Organic Acids

Genetics
Hormones, OATs & Genetics

• Hormones
  • Androgens, Estrogen, Progesterone, Adrenal

• Genetics
  • SNPs

• Organic Acids
  • Functional tests for nutrient deficiency and much more
How do the three come together?

• Hormones
• Genetics
• Organic Acids

• Each have independent value but can also compliment each other
Function (not “theoretical”) Medicine

Some Single Nucleotide Polymorphisms (SNPs) have not been proven to have negative consequences.

Some organic acids have theoretical connections that have not been proven.
How do genetics impact hormones?

Genetic defects (SNPs) relevant to Integrative Medicine practices often center on abnormal enzyme activity for detoxification via phase I or II metabolism.
Estrogen and Breast Cancer

The relationship between estrogen, its metabolism and breast (as well as others) cancer risk highlights the synergy between hormones and genetics.
Estrogen’s production, metabolism and clinical effects involve all 3, especially as it relates to cancer prevention.
Estrogen-related Cancer (Breast, etc.)

• Hormones
  • Hormone levels
  • Phase I metabolism (2-OHE1, 4-OHE1, 16-OHE1)
  • Phase II (methylation, glutathione, etc.)

• Genetics
  • CYP-1A1, 1B1, MTHFR, COMT & many more

• Organic Acids (markers of deficiency)
  • B6, B12, Folate, Glutathione, etc.
Estrogen Metabolism

- **Phase I**
  - 2,4 & 16-Hydroxylation
  - CYP – 1A1, 1B1, 3A4
- **Phase II**
  - Methylation
  - Glutathione, NAC
  - Glucuronidation, Sulfation
ESTROGENS: PHASE I METABOLISM
ESTROGENS: PHASE I METABOLISM
BEFORE & AFTER D.I.M.
A word about DIM and 2/16 ratio

- DIM/I3C increase CYP1A1 (2OH)
  - 2OH-Estrogens are “safer” than 4OH
- Estrogen metabolism matters, but the 2/16 ratio is not strongly associated with BC risk
- DIM increases 2-OH and reduces E1, E2 and usually 16-OHE1 and sometimes 4-OHE1
- Giving DIM to estrogen deficient women will exacerbate their low estrogen (bone loss?)
The other side of the DIM story

• DIM/I3C increase CYP1A1 (2OH)
• Common CYP1A1 SNPs also increase 2OH
• These SNPs are associated with INCREASED cancer risks
  • Toxins (PAH) can be worse with 2OH
• Think twice before giving to smokers
• DIM can work great, but don’t give DIM indiscriminately
How do estrogen metabolites “cause” Breast Cancer?

DNA Adducts

4-OHE$_1$(E$_2$)-1-N3Ade

Depurinating Adducts
How do estrogen metabolites “cause” Breast Cancer?

*N*-Acetylcysteine blocks formation of cancer-initiating estrogen-DNA adducts in cells
Breast Cancer, Estrogen-DNA Adducts

Figure 2. Depurinating estrogen-DNA adducts in the urine of healthy control women, women at high risk for breast cancer, and women with breast cancer. The ordinate of this bar graph corresponds to the ratio of depurinating DNA adducts divided by their respective estrogen metabolites, and thiol and methyl conjugates.
Catechol-estrogens that damage DNA are the “bad” guys. There are many steps along the way that encourage or discourage the formation of CE-DNA adducts. Nutrients and genetics impact many of these steps.
Red text=Moving towards trouble
Genetically defective or nutrient deficient enzymes can increase breast cancer risks via estrogen metabolites

Genetics and nutrient deficiencies can “move” the hormones to increase breast cancer risk

Cavalieri and Rogan Clin Trans Med (2016) 5:12
How do genetics impact Estrogen?

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Genetic Variation</th>
<th>Your Result</th>
<th>Gene Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Msp1 T&gt;C</td>
<td>TT</td>
<td>♂</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>A&gt;G Ile462Val</td>
<td>AA</td>
<td>♂</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>C&gt;G Val432Leu</td>
<td>GC</td>
<td>♀</td>
</tr>
<tr>
<td>CYP17A</td>
<td>34 T&gt;C</td>
<td>CC</td>
<td>♀</td>
</tr>
<tr>
<td>MnSOD</td>
<td>47 T&gt;C Ala16Val</td>
<td>TC</td>
<td>♀</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Insertion/Deletion</td>
<td>Deletion</td>
<td>♂</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Insertion/Deletion</td>
<td>Deletion</td>
<td>♀</td>
</tr>
<tr>
<td>COMT</td>
<td>472 G&gt;A (Val158Met)</td>
<td>AG</td>
<td>♀</td>
</tr>
<tr>
<td>MTHFR</td>
<td>677 C&gt;T</td>
<td>CC</td>
<td>♂</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>638 G&gt;A Arg213His</td>
<td>AA</td>
<td>♀</td>
</tr>
<tr>
<td>NQ01</td>
<td>609 C&gt;T</td>
<td>CC</td>
<td>♂</td>
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</tbody>
</table>
Multiple single nucleotide polymorphisms (SNPs) have been identified that can impact estrogen metabolism. These SNPs can change the activity of enzymes involved in estrogen metabolism. The table below shows some examples of SNPs and their impact on estrogen metabolism:

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<tr>
<td></td>
<td>A&gt;G Ile462Val</td>
<td>AA</td>
<td>2</td>
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<tr>
<td></td>
<td>C&gt;G Val432Leu</td>
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<td>3</td>
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<td></td>
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<td>4</td>
</tr>
<tr>
<td></td>
<td>Deletion</td>
<td>TC</td>
<td>5</td>
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<td></td>
<td>Deletion</td>
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<td>AG</td>
<td>7</td>
</tr>
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<td></td>
<td></td>
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<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>9</td>
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</tbody>
</table>

Single Nucleotide Polymorphism (SNP) Changes the enzyme activity.
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**Good News!**

**Bad News!**
**Table 4.** Combined effects of three genotypes (CYP1B1, COMT, GSTP1, and MnSOD) and risk of breast cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases/controls</th>
<th>OR (95% CI)*</th>
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<tbody>
<tr>
<td>CYP1B1 CC</td>
<td>COMT GG MnSOD TT</td>
<td>1/6 1.0</td>
</tr>
<tr>
<td>CYP1B1 CC</td>
<td>COMT GG MnSOD TC/CC</td>
<td>9.4 (1.1-80.4)</td>
</tr>
<tr>
<td>CYP1B1 CC</td>
<td>COMT GA/AA MnSOD TT</td>
<td>76/49 9.4 (1.1-80.4)</td>
</tr>
<tr>
<td>CYP1B1 CG/GG</td>
<td>COMT GG MnSOD TT</td>
<td>240/112 13.0 (1.5-109.0)</td>
</tr>
<tr>
<td>CYP1B1 CG/GG</td>
<td>COMT GA/AA MnSOD TT</td>
<td>206/102 12.2 (1.4-102.3)</td>
</tr>
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</table>
**Genetic Synergy in Breast Cancer**

**Table 4.** Combined effects of three genotypes (CYP1B1, COMT, GSTP1, and MnSOD) and risk of breast cancer

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<td>GG</td>
<td>TT</td>
</tr>
<tr>
<td>CG/GG</td>
<td>GA/AA</td>
<td>TC/CC</td>
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</tbody>
</table>
Genetics and nutrient deficiencies can “move” the hormones to increase breast cancer risk

Genotype

**CYP1B1**  **COMT**  **MnSOD**

- CYP1A/3A
- CYP 19 (aromatase)
- 4-androstene-3,17-dione
- Estradiol = E2
- Estrone = E1
- Testosterone

**CYP1B1 increases 4-OHE**

- 3x
- 3x

- COMT decreases methylation
- SOD decreases detox
Cancer Inhibition Strategies

- NAC

or covalently bind with the quinones to form nontoxic conjugates. Towards this goal, in a preliminary in vitro study, we have tested several antioxidants, NAcCys, GSH, cysteine (Cys), melatonin, resveratrol and reduced lipoic acid [35]. NAcCys was found to be one of the best inhibitors of the formation of depurinating adducts in reaction mixtures containing E2-3,4-Q or enzyme-activated 4-OHE₂ and DNA.

Published in final edited form as:

N-Acetylcysteine blocks formation of cancer-initiating estrogen-DNA adducts in cells
Muhammad Zahid¹, Muhammad Saeed¹, Mohammed F. Ali¹, Eleanor G. Rogan¹,², and Ercole L. Cavalieri¹,²,*
NAC Supplementation Lowers DNA Adducts

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

• NAC

Effect of NAcCys on 4-OHE$_2$ metabolism and formation of DNA adducts in MCF-10F and E6 cells treated with 4-OHE$_2$

Analysis of the culture media from MCF-10F cells treated with 1, 10 or 30 μM 4-OHE$_2$ alone showed an increase of 4-methoxycatechols, quinone conjugates and depurinating DNA adducts in a dose-dependent manner (Table 3, Fig. 4). In the presence of NAcCys, there was an increase in 4-methoxylation (Fig. 4A) and in quinone conjugates (4-OHE$_2$-2-SG, 4-OHE$_2$-2-Cys and 4-OHE$_2$-2-NAcCys) (Table 3). The depurinating adduct, 4-OHE$_2$-1-N$^3$Ade and 4-OHE$_2$-1-N$^7$Gua, levels were reduced 46% and 37%, respectively, with NAcCys present at half the concentration of 4-OHE$_2$. Their levels were reduced 86% and 85%, respectively, with equimolar NAcCys and 4-OHE$_2$. 
Cancer Inhibition Strategies
Lifestyle matters too (food, etc.)

Published in final edited form as:

Associations between Dietary Intake of Fruits and Vegetables in relation to Urinary Estrogen DNA Adduct Ratio

Kerryn W. Reding*,1,2, Muhammad Zahid3,*, Ercole Cavalieri3, Eleanor G. Rogan3, Brianne S. Raccor1, Charlotte Atkinson4, Mellissa Yong5, Katherine M. Newton6, and Johanna W. Lampe1,2
Our findings of an inverse association between EDA and botanical groups are supported by several lines of research. This includes: 1) reports of phytochemicals inducing enzymes which inactivate CEs, rendering them more easily excreted [12–14, 17, 20], including induction of GST enzymes by anthocyanidins (a flavonoid contained in plant-based foods, including berries, beets, bananas) [21, 22]; 2) inhibition of CE-activating enzyme, CYP1B1, by resveratrol as demonstrated in MCF-10F cells [16] and by berry supplementation in animal models [20]; and 3) phytochemicals, including resveratrol, inducing UGT activity [23]. Past studies have also reported on inverse associations between estrogen metabolites and fruit and vegetable intake in population-based studies [16, 24–27]. Overall, our findings are in general agreement with prior experimental work in this research area showing that resveratrol and NAcCys can inhibit both the formation of CE quinones and their reaction with DNA in cultured cells [14, 16, 17].

NAcCys, a potent antioxidant, can block quinone induced oxidative DNA damage through three primary mechanisms [28]. First, it can covalently bind with CE quinone to form an inactive quinone conjugate (4-OHE2-2- NAcCys). Second, it may act as a quencher of CE semiquinones (CE-SQ) that are generated during redox cycling between CE and quinones [28, 29]. Lastly and more importantly, through a biosynthesis pathway it can be transformed to cysteine, which could elevate the levels of cellular glutathione and produce a new NAcCys [30]. Resveratrol (3,5,4'-hydroxystilbene), present in grapes and other plants, has several anti-carcinogenic properties [31]. Resveratrol’s anticarcinogenic effect within the estrogen pathway is exhibited through the following three mechanisms. One is the induction of key protective enzymes (NQ01) in estrogen metabolism pathway that provides a decrease in CE quinone and corresponding increase in CE concentrations [16]. The second mechanism is the modulation of activating enzyme CYP1B1, which decreases the formation of 4-CE [16]. The third is through reduction of CE-SQ to CE, as indirectly determined in vitro.
Supplement Intervention

Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician’s Expectation Be Matched by the Reality?
Christine A. Houghton, Robert G. Fassett, and Jeff S. Coombes

concentration of a compound required to double the activity of the Phase II detoxification enzyme, quinone reductase [83, 87–89, 91].
Sulforaphane increases Quinone Reductase
Cancer Inhibition Strategies

Support Phase I Metabolism
  • NAC (also supports phase II)
  • DIM (or I3C)
  • Resveratrol, Sulforaphane

Support Phase II Metabolism
  • Glutathione
  • Sulfur
  • Methylation support
Genetics and nutrient deficiencies can impact hormones in meaningful ways. Adequate intake of nutrients may be different if you have genetic defects. Organic Acids may help to reveal functional deficiencies.
Organic Acids

• Functional tests for nutrient deficiency
• Methylmalonate (MMA) is a good example
  • Well characterized example for B12 deficiency
  • When B12 is low, MMA is high
  • When B12 cannot get to the cell, MMA is high

The conversion of methylmalonyl coenzyme A to succinyl coenzyme A requires vitamin B$_{12}$; therefore, a deficiency of vitamin B$_{12}$ causes increases in the concentration of MMA (21). In fact, MMA concentrations often increase in early stages of vitamin B$_{12}$ deficiency before measurable decreases in serum vitamin B$_{12}$.

Clinical Chemistry 46:8(B) 1277–1283 (2000)
MMA – Functional B12 Deficiency Marker
When serum B12 is “normal” but cellular levels are low (in this case due to a genetic defect in the transport protein), MMA still increases.

Table 1. Characteristics of study sample by transcobalamin II genotype

<table>
<thead>
<tr>
<th>Transcobalamin II genotype</th>
<th>PP</th>
<th>PR</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>39</td>
<td>63</td>
<td>26</td>
</tr>
<tr>
<td>Methylmalonic acid (nM)</td>
<td>208 (± 96)</td>
<td>206 (± 80)</td>
<td>264 (± 138)‡</td>
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</tbody>
</table>

We conclude that the TCII 775G>C genotype significantly influences tissue B12 delivery and functional B12 status.
<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Related Organic Acids</th>
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</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>Methylmalonate</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Xanthurenonate, Kynurenonate</td>
</tr>
<tr>
<td>Folate</td>
<td>FIGLU</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Pyroglutamate</td>
</tr>
<tr>
<td>Nutrients</td>
<td>Related Organic Acids</td>
</tr>
<tr>
<td>----------------</td>
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</table>

NOT an exhaustive list
Nutrients

• Vitamin B12
• Vitamin B6
• Folate
• Glutathione

Relevant to Phase II metabolism of estrogens

- Needed for COMT to methylate estrogens
- Detoxifies estrogen
Not all organic acids are strong biomarkers

- Vitamin B12
- Vitamin B6
- Folate
- Glutathione
- Methylmalonate
- Xanthurenenate, Kynurenate
- FIGLU
- Pyroglutamate
Organic Acid Categories

- Vitamin Deficiency Markers
- Neurotransmitter metabolites
- Detoxification indicators
- Oxidative Damage
- Bacterial (gut) and yeast markers
- Fatty Acid Metabolism
- Carbohydrate Metabolism
- Energy Production Markers (CAC)
Citric Acid Cycle
# Citric Acid Cycle

<table>
<thead>
<tr>
<th>CITRIC ACID CYCLE METABOLITES</th>
<th>RESULT</th>
<th>POSSIBLE CAUSES</th>
<th>ADDITIONAL INVESTIGATIONS</th>
<th>TREATMENT CONSIDERATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citric Acid or Cis-Aconitic Acid</strong>&lt;br&gt;Metabolites of acetyl CoA; precursors of isocitric acid</td>
<td>High</td>
<td>• Impaired metabolism due to toxic metals (Fl, Hg, As)&lt;br&gt;• Low glutathione&lt;br&gt;• High amounts of dietary citric acid&lt;br&gt;• Metabolic acidosis (if mildly increased cis-aconitic acid but markedly increased citric acid)</td>
<td>• Comprehensive Urine Elements Profile&lt;br&gt;• FLDP&lt;br&gt;• CDSA</td>
<td>• Rule out toxic metals&lt;br&gt;• Glutathione</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>Low or high pyruvic acid or low acetylCoA (from fatty acid oxidation)</td>
<td>See notes for pyruvic acid</td>
<td>See notes for pyruvic acid</td>
<td></td>
</tr>
<tr>
<td><strong>Isocitric Acid</strong>&lt;br&gt;Metabolite of cis-aconitic acid; precursor of alpha-ketoglutaric acid</td>
<td>High</td>
<td>• Impaired metabolism due to low cofactors (B3, Mg, Mn)&lt;br&gt;• Aluminium toxicity</td>
<td>• Vitamins and Minerals Analysis&lt;br&gt;• Comprehensive Urine Elements Profile&lt;br&gt;• HMA</td>
<td>• Rule out Al toxicity&lt;br&gt;• B3, Mg, Mn</td>
</tr>
</tbody>
</table>
Citric Acid Cycle

Does high Isocitrate mean low B3, Mg, Mn?

Mg used in hundreds of metabolic steps
Citric Acid Cycle

Does high Isocitrate mean low B3, Mg, Mn?
High HMG = Low CoQ10?
Not all organic acids are strong biomarkers

- Vitamin B12
- Vitamin B6
- Folate
- Glutathione
- Methylmalonate
- Xanthurenate, Kynurenate
- FIGLU
- Pyroglutamate
Organic Acids and Hormones Collide Directly
Estrogen & low B6 leads to increased Xan & Decreased Insulin Sensitivity
The influence of oestrogens on tryptophan metabolism in man.

Author: Rose DP

Abstract: Urinary excretion of 3-hydroxykynurenine, xanthurenic acid and 3-hydroxyanthranilic acid following a tryptophan load (5 gm orally) was studied in 22 normal subjects, 12 women taking estrogen-progestogen preparations and 5 patients receiving estrogen alone. 3-hydroxykynurenine and 3-hydroxyanthranilic acid were measured by column chromatography and xanthurenic acid was determined by thin-layer chromatography. The excretions of 3-hydroxykynurenine and xanthurenic acid were high in both groups of hormone treated subjects. In some instances the 3-hydroxyanthranilic acid levels were also raised. Large doses of pyridoxine (20 mg orally twice daily for 6 days or 1 mg/day for 15 days then 20 mg following another tryptophan load plus another 20 mg pyridoxine) caused the high excretions to return to normal or near normal in the 3 subjects studied. It was suggested that these findings indicate an estrogen-induced increase in the capacity for conversion of tryptophan to nicotinic acid. As a result tryptophan loading produces a relative shortage of pyridoxal phosphate coenzyme, with a corresponding increase in the urinary excretion of 3-hydroxykynurenine and xanthurenic acid. (AUTHOR’S, MODIFIED)
Estrogen & low B6 leads to increased Xan & Decreased Insulin Sensitivity

**INSULIN AND THE KYNURENINES**

The kynurenine metabolites can potentially interfere with pancreatic function in several ways. For example, Kotake et al. (1) showed that xanthurenic acid combines easily with insulin to form a complex with reduced insulin activity. It is important to note that this complex formation would not lead to a reduced apparent concentration of insulin in plasma as the complex was antigenically indistinguishable from normal insulin (1). The same group indeed demonstrated that the administration of xanthurenic acid to rats produced a diabetic state (1).
Estrogen & low B6 leads to increased Xan & Decreased Insulin Sensitivity

Tryptophan → Kynurenine → Quinolinate (hepatic) → NAD

Serotonin → 5-HIAA

Estrogen +

Decreased Insulin Sensitivity
WHAT ELSE leads to Decreased Insulin Sensitivity and Serotonin?

Estrogen, Cortisol, Inflammation, LPS
How does stress “cause” Depression, Diabetes?

**Pathway:**
- **Tryptophan** → **Kynurenine**
- **Cortisol** activates the pathway
- **Kynurenine** → **Xanthurenic acid**
- **Xanthurenic acid** leads to decreased **Serotonin** and increased **5-HIAA**
- Insulin synthesis is affected by the pathway

**References:**
- BRITISH JOURNAL OF PSYCHIATRY (2002), 180, 99–100
  - Cortisol, serotonin and depression: all stressed out?
  - P. J. Cowen
A higher baseline CAR was associated with a significantly increased risk of developing MDD by follow-up. No other baseline cortisol measures were significant prospective predictors of MDD.

How does stress “cause” Depression, Diabetes?

Cortisol Awakening Response (CAR)
How does stress—“cause” Depression, Diabetes?

inflammation

Tryptophan → Kynurenine → Xanthurenate → Insulin

Serotonin → S-HIAA

Inflammation

Organic Acids

Neuroscience and Biobehavioral Reviews 36 (2012) 658-676

Depression and type 2 diabetes: Inflammatory mechanisms of a psychoneuroendocrine co-morbidity

Michael J. Stuart, Bernhard T. Baune
Altered tryptophan metabolism

- Estrogen
- Cortisol
- Inflammation
- LPS (gram negative bacteria)
Organic Acids can compliment Hormone Analysis

Genetics can compliment both
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>What does it affect</th>
<th>Result of Defect</th>
<th>Treatment Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Phase I Metabolism</td>
<td>Defects INCREASE Enzyme Activity</td>
<td>Reduce exposure to toxins (PAHs, aromatic amines, nitrates,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>smoking). Optimize phase II metabolism. DIM may be contraindicated with CYP1A1.</td>
</tr>
<tr>
<td>CYP1A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP17A</td>
<td>Hormone Production</td>
<td>Defect INCREASES E2</td>
<td>Test estrogens, dietary fiber, calcium-d-glucurate</td>
</tr>
<tr>
<td>MnSOD</td>
<td>Cell Antioxidant Activity</td>
<td>Defect is protective</td>
<td>Fruit/Veggie, Antioxidants, Mn, Low-Moderate intensity Exercise</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Glutathione Detox</td>
<td>Gene deletions increases risks for various cancers</td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td>Avoid toxins, increase antioxidants and crucifers</td>
</tr>
<tr>
<td>COMT</td>
<td>Methylation</td>
<td>3-4x reduced activity</td>
<td>B12, folate, Mg, Sulforphane</td>
</tr>
<tr>
<td>MTHFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SULT1A1</td>
<td>Inactivates Estrogen</td>
<td>Inactivates Estrogen</td>
<td>Active folate, B-vitamins, SAMe</td>
</tr>
<tr>
<td>NQ01</td>
<td>Quinone Reductase</td>
<td>TT Variant &lt;5% activity</td>
<td>Sulphorophane, reduce toxins</td>
</tr>
</tbody>
</table>

**What does it affect**

- Phase I Metabolism
- Hormone Production
- Cell Antioxidant Activity
- Glutathione Detox
- Methylation
- Inactivates Estrogen
- Quinone Reductase

**Result of Defect**

- Defect INCREASE Enzyme Activity
- Defect INCREASES E2
- Defect is protective
- Gene deletions increases risks for various cancers
- 3-4x reduced activity
- TT= 3x reduced activity
- Inactivates Estrogen
- TT Variant <5% activity

**Treatment Considerations**

- Reduce exposure to toxins (PAHs, aromatic amines, nitrates, smoking). Optimize phase II metabolism. DIM may be contraindicated with CYP1A1.
- Test estrogens, dietary fiber, calcium-d-glucurate
- Fruit/Veggie, Antioxidants, Mn, Low-Moderate intensity Exercise
- Avoid toxins, increase antioxidants and crucifers
- B12, folate, Mg, Sulforphane
- Active folate, B-vitamins, SAMe
- Sulphorophane, reduce toxins
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Lab Tests</th>
<th>What does it affect</th>
<th>Result of Defect</th>
<th>Treatment Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Estrogens and Metabolites (2-OHE, 4-OHE, 16-OHE)</td>
<td>Phase I Metabolism</td>
<td>Defects INCREASE Enzyme Activity</td>
<td>Reduce exposure to toxins (PAHs, aromatic amines, nitrates, smoking). Optimize phase II metabolism. DIM may be contraindicated with CYP1A1.</td>
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<td>CYP1B1</td>
<td>Hormone Production</td>
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<td>COMT</td>
<td>Quinone Reductase</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MTHFR</td>
<td>E1, E2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Methylation – Hormones, Genetics and OATs

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<th>Lab Tests</th>
<th>What does it affect</th>
<th>Result of Defect</th>
<th>Treatment Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>Estrogen Metabolites, OATS</td>
<td>Methylation</td>
<td>3-4x reduced activity</td>
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</tr>
<tr>
<td>MTHFR</td>
<td></td>
<td>Methylation</td>
<td>TT= 3x reduced activity</td>
<td>Active folate, B-vitamins, SAMe</td>
</tr>
</tbody>
</table>
**Genetics – Does it matter?**

<table>
<thead>
<tr>
<th>Gene &amp; Variation</th>
<th>rsID</th>
<th>Alleles</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT V158M</td>
<td>rs4680</td>
<td>AA</td>
<td>+/-</td>
</tr>
<tr>
<td>COMT H62H</td>
<td>rs4633</td>
<td>TT</td>
<td>+/-</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>rs1801133</td>
<td>GG</td>
<td>-/</td>
</tr>
<tr>
<td>MTHFR 03 P39P</td>
<td>rs2066470</td>
<td>GG</td>
<td>-/</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>rs1801131</td>
<td>TT</td>
<td>-/</td>
</tr>
</tbody>
</table>

- 3-4 fold decreased COMT activity
- Not deficient in methyl donors
Why Poor Methylation?

Genetic

• MTHFR – methyl donor deficient
• COMT – methylation enzyme defunct

Nutrient Deficient

• Vitamin B12 (Methylmalonate high?)
• Vitamin B6 (Xanthurenenate high?)
• Folate (FIGLU high?)
• Magnesium
General Methylation/COMT Support

- Magnesium
- SAMe
- Trimethyl glycine (TMG)
- Choline
- Methionine
- Folate
- B-6 (P5P – Pyridine-5-Phosphate)
- B-12 (different forms)
Inhibition of catechol-O-methyltransferase increases estrogen-DNA adduct formation

Abstract

The association found between breast cancer development and prolonged exposure to estrogens suggests that this hormone is of etiologic importance in the causation of the disease. Studies on estrogen metabolism, formation of DNA adducts, carcinogenicity, cell transformation and mutagenicity have led to the hypothesis that reaction of certain estrogen metabolites, predominantly catechol estrogen-3,4-quinones, with DNA forms depurinating adducts [4-OHE\(_1\)(E\(_2\))-1-N3Ade and 4-OHE\(_1\)(E\(_2\))-1-N7Gua]. These adducts cause mutations leading to the initiation of breast cancer. Catechol-O-methyltransferase (COMT) is considered an important enzyme that protects cells from the genotoxicity and cytotoxicity of catechol estrogens, by preventing their conversion to quinones. The goal of the present study was to investigate the effect of COMT inhibition on the formation of depurinating estrogen-DNA adducts. Immortalized human breast epithelial MCF-10F cells were treated with 4-OHE\(_2\) (0.2 or 0.5 \(\mu\)M) for 24 h at 120, 168, 216, and 264 h post-plating or one time at 1–30 \(\mu\)M 4-OHE\(_2\) with or without the presence of COMT inhibitor (Ro41-0960). The culture media were collected at each point, extracted by solid-phase extraction and analyzed by HPLC connected with a multichannel electrochemical detector. The results demonstrate that MCF-10F cells oxidize 4-OHE\(_2\) to E\(_1\)(E\(_2\))-3,4-Q, which react with DNA to form the depurinating N3Ade and N7Gua adducts. The COMT inhibitor Ro41-0960 blocked the methoxylation of catechol estrogens, with concomitant 3–4 fold increases in the levels of the depurinating adducts. Thus, low activity of COMT leads to higher levels of depurinating estrogen-DNA adducts that can induce mutations and initiate cancer.
Inhibition of catechol-O-methyltransferase increases estrogen-DNA adduct formation

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How do estrogen metabolites “cause” Breast Cancer?
Other tests run in parallel that play a role in BC

- Melatonin
- 8-OHdG
- Cortisol
- Thyroid (blood)
- Stool/SIBO
- Vitamin D (blood)
- And the list goes on
Low Melatonin=Higher BC Risk

Table 2. Odds ratios (ORs) and 95% confidence intervals of breast cancer by quartile of urinary 6-sulphatoxymelatonin (aMT6s) level*  

<table>
<thead>
<tr>
<th>Group and parameter</th>
<th>Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Urinary aMT6s, ng/mg creatinine</td>
<td>&lt;11.5</td>
</tr>
<tr>
<td>Invasive breast cancer cases</td>
<td></td>
</tr>
<tr>
<td>No. of case patients/No. of control subjects</td>
<td>50/73</td>
</tr>
<tr>
<td>Invasive and in situ breast cancer cases</td>
<td></td>
</tr>
<tr>
<td>No. of case patients/No. of control subjects</td>
<td>61/94</td>
</tr>
</tbody>
</table>

These prospective data support the hypothesis that higher melatonin levels, as measured in first morning urine, are associated with a lower risk of breast cancer. [J Natl Cancer Inst 2005;97:1084–7]
Conclusion: Low levels of endogenous melatonin production among older individuals may lead to higher levels of oxidatively damaged guanine in DNA, thereby possibly increasing the risk of developing cancer.
Association between Urinary Excretion of Cortisol and Markers of Oxidatively Damaged DNA and RNA in Humans

Abstract

Chronic psychological stress is associated with accelerated aging, but the underlying biological mechanisms are not known. Prolonged elevations of the stress hormone cortisol is suspected to play a critical role. Through its actions, cortisol may potentially induce oxidatively generated damage to cellular constituents such as DNA and RNA, a phenomenon which has been implicated in aging processes. We investigated the relationship between 24 h excretion of urinary cortisol and markers of oxidatively generated DNA and RNA damage, 8-oxo-7,8-dihydro-2'-deoxyguanosine and 8-oxo-7,8-dihydroguanosine, in a sample of 220 elderly men and women (age 65 – 83 years). We found a robust association between the excretion of cortisol and the oxidation markers ($R^2 = 0.15$, $P<0.001$ for both markers). Individuals in the highest quartile of cortisol excretion had a 57% and 61% higher median excretion of the DNA and RNA oxidation marker, respectively, than individuals in the lowest quartile. The finding adds support to the hypothesis that cortisol-induced damage to DNA/RNA is an explanatory factor in the complex relation between stress, aging and disease.
Association between Urinary Excretion of Cortisol and Markers of Oxidatively Damaged DNA and RNA in Humans

- 8-oxodG
- $R^2 = 0.15$, $P < 0.001$
Prioritizing Testing

• Hormones
• Genetics
• Organic Acids

• Each have independent value but can also compliment each other
Prioritizing Testing

More comprehensive testing can change the direction of treatment. The more we understand the relationships between biomarkers and genetics, the more we can answer the “why” question.
Standard Testing: CBC, CMP, Lipids, etc.

Basic Hormones (Thyroid, Sex, Adrenal, Vit D)

G.I., Advanced Hormones

Organic Acids

Genetics
Prioritizing Testing
The relative value of one test over another really depends on what other information you value in your practice and especially in a particular case.
Prioritizing Testing

The value of testing can be increased by better understanding the informational synergy these labs can provide when their relationships are understood.
Hormones, Organic Acids and Genetics: Clinical Synergy for Integrative Medicine

THANK YOU FOR LISTENING!

Mark Newman, MS
President, Precision Analytical
newman.testing@gmail.com