Methylation and Estrogens

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Whole Detox Webinar
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“Clinicians will be central to helping consumer-patients use genomic information to make health decisions.” – NEJM
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President and CEO of:
• Seeking Health Educational Institute (www.seekinghealth.org)
• Seeking Health (www.seekinghealth.com)
• MTHFR.Net (www.mthfr.net)
How do Genes work?
The MTHFR gene produces the MTHFR enzyme.
Pathway Planner
Biopterin
Version 2.0

SeekingHealth.org

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Methylation
Methylation

Functions of Methylation (some):
- Gene regulation: turn on/off genes via SAMe
- Biotransformation: glutathione production
- DNA base formation: uracil $\rightarrow$ thymine
- Cell membrane components: phosphatidylcholine and DHA to membranes
- Mitochondrial support: adenosine as substrate for ATP

Estrogen

**Essential Hormone**

- Bone mass
- Female reproduction
- Skin health
- Cardiovascular health

Estrogen Types

- Estrone (E1) – produced by ovaries – main form post-menopause
- Estradiol (E2) – produced by ovaries and levels vary with menstrual cycle
- Estriol (E3) – only during pregnancy – produced by placenta
Estrogen Types - Xenoestrogens

- Herbicides – Atrazine
- Insecticides - Endosulfan
- BPA
- Pthalates
- PCB and DDT (banned but still around)
- DDT
- Dioxins – released during combustion – get into animal fat
Uteroplacental aromatase, cytochrome P450 s and catechol-O-Methyltransferase activities
↓ Fetal 16-α and 17-α hydroxylase activity

Aberrant levels of primary estrogens and estrogen metabolites

↓ NO  
↓ PGI₂  
↑ TXA₂  
↑ ET

↓ VEGF  
↓ PIGF  
↑ sFlt  
↑ sEng

↑ ROS  
↑ Lipid peroxides  
↑ Superoxide  
↑ HIF-1α

↑ TNFα  
↑ IL-6  
↑ IL-8

↓ Vasodilatation  
↓ Angiogenesis  
↑ Oxidative stress/hypoxia  
↑ Inflammation

↓ Cytotrophoblast invasion  
↓ Uteroplacental perfusion  
↑ Placental ischemia

Clinical manifestation of preeclampsia
On the Sulfation and Methylation of Catecholestrogens in Human Mammary Epithelial Cells and Breast Cancer Cells

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Prolonged exposure to high level of estrogen is a known risk factor for breast carcinogenesis. In cells, estrogen, in particular estrone (E1) and 17β-estradiol (E2), can be converted to catecholestrogens (CEs) which may be oxidized to form CE-semiquinones and CE-quinones that are capable of binding to DNA to induce mutations, followed by carcinogenesis. Whether the body is equipped with protective mechanisms against potentially harmful CEs, therefore, is an important issue. The present study was designed to examine the role of sulfation in the metabolism of CEs. MCF-7 breast cancer cells and MCF 10A human mammary epithelial cells were metabolically labeled with [35S]sulfate in the presence of individual CEs. Analysis of the labeling media showed the generation and release of exclusively [35S]sulfated 2-methoxy-E1 or [35S]sulfated 2- or 4-methoxy-E2 by cells labeled in the presence of 2-OH-E1 or 2- or 4-OH-E2. Whereas both [35S]sulfated 4-methoxy-E1 and [35S]sulfated 4-OH-E1 were detected in the labeling media of cells labeled in the presence of 4-OH-E1. These results indicated a concerted action of catechol-O-methyltransferase (COMT) and the cytosolic sulfotransferase (SULT) enzyme(s) in the metabolism of CEs. Enzymatic assays revealed that, five (SULT1A1, SULT1A2, SULT1A3, SULT1C4, and SULT1E1) of eleven known human SULTs tested could use CEs and methoxyestrogens (MEs) as substrates, with SULT1E1 displaying the strongest sulfating activity.

Key words catecholestrogen; sulfation; methylation; methoxyestrogen; cytosolic sulfotransferase
**Figure S1:** Part of the metabolic pathway of estradiol and the role of various enzymes involved: Estradiol is metabolized into 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2) by CYP1A1 and CYP1B1 respectively. These catechols undergo further oxidation into semiquinones and quinones that react with DNA to form depurinating adducts leading to mutations associated with breast cancer. NQO1 reduces these quinones back to catechols which are detoxified into methoxy derivatives by the action of COMT. This protects the cells against DNA adducts formation and lowers the potential for mutagenic damage.
5α-metabolism makes androgens more androgenic, most notably 5α-DHT is the most potent testosterone metabolite (~3x more potent than testosterone itself). 5α-Reductase activity is assessed using the ratio of Androsterone (5α) to Etiocicholanolone (5β).

If not methylated, 4-OH-E1 can bind to and damage DNA.
COMT Downregulation: Symptoms Match SNP?

What is issue? ↑ Dopamine? ↑ Norepi? ↓ Epi? ↑ Xeno/Estrogen?

Support COMT:
- Molybdenum (prn)
- Magnesium
- Vitamin B6
- Vitamin C
- Niacin
- SAM
- Adaptogens
- Watch Phenylalanine and Tyrosine Intake
- MSM, NAC or sulfur-containing foods (if tolerated)
- Blood sugar stabilization (diet, sleep, exercise, chromium)
Breast cancer is the most common malignancy and a major cause of mortality in women worldwide [1]. Accumulating evidence indicates that an increased risk for breast cancer is associated with dietary factors [2] and a few significant-risk genetic components (e.g., BRCA1) [3]. BRCA1 is a tumor suppressor gene which plays a key role in numerous cellular processes, including transcription regulation, DNA damage repair and protein ubiquitination.4 Recent research has confirmed that BRCA1 is an important transcriptional regulator, and BRCA1-depleted breast cancer cells shows changes to approximately 7% of the mRNAs expressed [4]. Moreover, our recent study also indicated that Phosphatidylethanolamine N-methyltransferase (PEMT) is a small integral membrane protein that catalyzes the de novo synthesis of choline using S-adenosylmethionine as a methyl donor [17]. The human PEMT gene is located on factor receptor displayed different expression patterns in BRCA1-defective cancer cells [5,6], and confirmed that differential epigenetic regulation of transcription exist along with BRCA1 inactivation [7,8]. Therefore, one can speculate that there are wide ranges of gene expression and regulation differences between BRCA1 dysfunction and the basal phenotype. To date, choline is among the well-studied essential nutrients that are involved in breast cancer; for example: (i) choline-containing compounds are significantly changed in breast cancer [9,10]; (ii) choline intake is inversely correlated with breast cancer risk [11-13]; and (iii) aberrant choline metabolism is often associated with malignant transformation, invasion, and metastasis of breast cancer [14-16]. Epigenetic change-mediated abnormal PEMT expression in BRCA1-mutated breast cancer progression.

FIGURE 1. Major reactions involved in transmethylation flux and methylome generation. The total transmethylation flux is equivalent to the total flux occurring through reactions that convert S-adenosylmethionine to S-adenosylhomocysteine. The 3 S-adenosylmethionine-dependent reactions thought to contribute quantitatively most to this flux are methylation of guanidinoacetate by guanidinoacetate methyltransferase (GAMT) to form creatine; methylation of phosphatidylethanolamine by phosphatidylethanolamine methyltransferase (PEMT) to form phosphatidylcholine; and methylation of glycine by glycine N-methyltransferase (GNMT) to form sarcosine (N-methylglycine). A large number of additional S-adenosylmethionine-dependent methyltransferases also occur in mammals [see Katz et al (3)], but their collective quantitative contribution to transmethylation flux may be small compared with those mentioned above. The final steps in methylome generation are the reduction of a methylene group of 5,10-methylenetetrahydrofolate (methylene-THF) by methylenetetrahydrofolate reductase (MTHFR) to form 5-methyltetrahydrofolate (methyl-THF), followed by transfer by methionine synthase of the newly formed methyl moiety to homocysteine, forming methionine and tetrahydrofolate (THF). Sarcosine is formed not only by GNMT, but also by oxidation of choline to betaine, formation of dimethylglycine by betaine homocysteine methyltransferase (BHMT), and oxidation of dimethylglycine to sarcosine. Sarcosine is oxidized by sarcosine dehydrogenase (SDH). During the reaction, glycine is produced, and a 1-carbon unit is transferred to THF, forming methylene-THF. MAT, methionine adenosyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase; SAHH, S-adenosylhomocysteine hydrolase.
Arsenic and Cancer

Conditions related to arsenic-induced toxicity
- Skin conditions
- CVD
- PVD
- Lung disorders
- Neurological disorders

Arsenic increases risk of cancer (skin, lung, bladder, liver, kidney) via multiple routes
- Various enzyme inhibition (SUOX, PDH, etc)
- ↓ DNA repair
- Chromosomal instability
- ↑ ROS (Superoxide – needing SOD & H₂O₂ – needing GSH and CAT)
- ↓ DNA methylation
- Altered gene expression
Arsenic Exposures

Increased arsenic levels due to:

- Drinking water
- Pesticides
- Antibiotics (in chicken)
- Rice
- Apples
- Sea vegetables
- SNPs
Figure 1. The metabolism pathway of inorganic arsenic showing arsenate reduction to arsenite and methylation to pentavalent and trivalent forms.

2. Genotoxicity

The genotoxic role of iAs in the cells has long been controversial. Arsenic is reported to cause DNA modifications such as aneuploidy, micronuclei formation, chromosomal aberrations, deletion mutations, sister chromatid exchange and DNA-protein cross-linking [1]. Several mechanisms have been proposed to explain the genotoxicity of arsenic, as well as induction of oxidative stress and altered patterns of DNA repair [15].
Action Steps
Lab Testing

Lab Tests (Routine labs plus...)

- Fasting insulin
- Fatty Liver Index - [http://www.mayoclinic.org/medical-professionals/model-end-stage-liver-disease/alcoholic-liver-disease-nonalcoholic-fatty-liver-disease-index](http://www.mayoclinic.org/medical-professionals/model-end-stage-liver-disease/alcoholic-liver-disease-nonalcoholic-fatty-liver-disease-index)
- Hormone testing
- VAP – advanced cholesterol panel
- Oxidative Stress – lipid peroxidation, SOD and GPX expression
- Methylation profile
- RBC Fatty Acids
- Pathogens – viral, mold, bacterial, Lyme, parasites
- Lactate
- Electrolytes
- Environmental – heavy metals (unprovoked and provoked – inc post-chemo)
- Genetic Testing (23andMe, et al)
Prevention, Treatment and Follow Up

Lifestyle, Diet and Other Recommendations

- Belly breathing
- Watch intake with carbs and sugars
- Support mitochondrial respiration
- Sauna
- Eat to 80% full
- Intermittent fasting
- Meditation
- Appropriate Exercise
- Earthing
- Raw foods and vegetable/berry/greens juicing with pulp ➔ ↑ e-
### Methylation Profile: plasma

<table>
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<tr>
<th>PRIMARY &amp; INTERMEDIATE METABOLITES</th>
<th>RESULT/UNIT</th>
<th>REFERENCE INTERVAL</th>
<th>2.5&lt;sup&gt;th&lt;/sup&gt;</th>
<th>16&lt;sup&gt;th&lt;/sup&gt;</th>
<th>50&lt;sup&gt;th&lt;/sup&gt;</th>
<th>84&lt;sup&gt;th&lt;/sup&gt;</th>
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<tr>
<td>Methionine</td>
<td>2.0 µmol/dL</td>
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<td>Cysteine</td>
<td>50 µmol/dL</td>
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<td>S-adenosylmethionine (SAM)</td>
<td>205 nmol/L</td>
<td>86 - 145</td>
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<td>S-adenosylhomocysteine (SAH)</td>
<td>46.1 nmol/L</td>
<td>10 - 22</td>
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<td>Homocysteine</td>
<td>20.6 µmol/L</td>
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<td>Cystathionine</td>
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### Methylation Index

<table>
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<tr>
<th>METHYLATION INDEX</th>
<th>RESULT</th>
<th>REFERENCE INTERVAL</th>
<th>PERCENTILE</th>
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<td>SAM : SAH</td>
<td>4.5</td>
<td>&gt; 4</td>
<td>68&lt;sup&gt;th&lt;/sup&gt;</td>
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</table>
Environmental Factors Affecting the Epigenome

Epigenetic Modulation: Methylation Deficiency

Damaged nDNA & mtDNA

Gene Instability
- DNA Damage
- Purine / Pyrimidine Deficiency

Development of Epigenetic Pathologies & Disease

Altered Gene Expression
- Low SAM: SAH Ratio
- Hypermethylation CpG islands
- Hypomethylation CpG sites

Adapted From:
DNA Methylation in the Inflammatory Response and Relevance to Chronic Kidney Disease

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Thank You

Great ways to stay informed:

• Free Methylation Video Available at www.SeekingHealth.org
• Facebook: https://www.facebook.com/drbenjaminlynch
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• Nutrigenomic Conference Recordings – www.SeekingHealth.org
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