New Insights into the Mechanism of Action of Antioxidants

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Dietary Antioxidant

A substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans.

Dietary Reference Intakes, Foods and Nutrition Board
2000 Natl Acad Press
Reactive Species

Includes:
- hydroxyl radicals (·OH)
- superoxide anions (O$_2^-$)
- singlet oxygen (¹O$_2$)
- hydrogen peroxides (H$_2$O$_2$)
- organic peroxides (R-OOH)
- nitric oxide
- peroxynitrite
Generation of Reactive Oxygen Species

- Reticuloendothelium
  - O$_2$
  - NADPH oxidase
  - P450 oxidase
  - Xanthine oxidase

- Peroxisome
  - Cu,Zn Superoxide Dismutase
  - oxidase
  - H$_2$O$_2$
  - GSH
  - Fe$^{+2}$/Cu$^+$

- Mitochondria
  - O$_2$
  - H$_2$O$_2$
  - H$_2$O

- Glutathione Peroxidase
  - GSSG
  - GSH

- Catalase
  - Thioredoxin reductase
  - Fe$^{+3}$/Cu$^{+2}$

- $\cdot$OH
Nitric Oxide Dependent Reactions

O$_2^-$

NO (nitric oxide)

Peroxynitrite

Oxidation eg GSSG

Nitrosation (NO) eg GSNO

Nitration (NO$_2$) NO2-tyrosine
Reactive Oxygen Species

- O$_2$ → H$_2$O$_2$ → •OH

- Damage DNA, RNA
- Oxidize Proteins (enzymes, histones)
- Oxidize Lipids
- Activate Cell Suicide
Diversity in Dietary Antioxidants

**Essential**
- Vitamin E (tocopherol)
- Vitamin C (ascorbic acid)
- Vitamin A (retinol and carotenoids)
- Numerous minerals- Cu, Mn, Zn, Se, Fe

**Non-essential**
- Glutathione, small peptides
- Host of phytochemicals (thousands in food supply)
In Vitro Measures of Antioxidant Capacity

• ABTS Assay for Antioxidant Activity (Miller et al. 1997).


• FOX3 (Lipid Peroxidation) Assay. (Hermes-Lima et al. 1995)

• Total Phenolics. (Spanos and Wrolstad, 1990).

• ORAC (Ou et al, 2001)
Relative ORAC Efficacy (FL Units)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Units</th>
<th>Fluids</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic Acid</td>
<td>4.37</td>
<td>Serum</td>
<td>347</td>
</tr>
<tr>
<td>Quercetrin</td>
<td>7.28</td>
<td>Urine</td>
<td>1542</td>
</tr>
<tr>
<td>Genistein</td>
<td>5.93</td>
<td>Blueberry Juice</td>
<td>23748</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.62</td>
<td>Grape Juice</td>
<td>31441</td>
</tr>
<tr>
<td>Catechin</td>
<td>6.76</td>
<td>Raspberry Juice</td>
<td>54034</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.95</td>
<td>Black Tea</td>
<td>17267</td>
</tr>
</tbody>
</table>

Ou et al, J Agric Food Chem 2001;49:4619-26
In Vitro Antioxidants Measurement Interpretation

• Different assays measure the effects of different radical species

• Not clear how to integrate test results for meaningful reflection of in vivo status

Biomarkers

Pathologic disorders → Xenobiotics → Free radicals $R^\cdot$

- Metabolic process
- $O_2^-$, $OH^\cdot$, $^{1}O_2$

- Oxidized nucleotides
  - 8-hydroxy guanosine

- Lipid peroxides
  - Alkanes
    - Ethane
    - Pentane

- Oxidated AA
  - 0-tyrosine
  - Dityrosine

- Malondialdehyde
Low or High VF Diets and 8-OHdG in Lymphocyte DNA

<table>
<thead>
<tr>
<th>Diet¹</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>residues/10⁶dG</td>
<td>10⁶dG</td>
<td></td>
</tr>
<tr>
<td>Low VF¹</td>
<td>10.1 ± 1.0</td>
<td>10.0 ± 0.9</td>
<td>-0.5</td>
</tr>
<tr>
<td></td>
<td>(8.4)</td>
<td>(9.4)</td>
<td></td>
</tr>
<tr>
<td>High VF</td>
<td>9.7± 1.2</td>
<td>8.1 ± 1.0*</td>
<td>-16.5</td>
</tr>
<tr>
<td></td>
<td>(7.8)</td>
<td>(5.2)</td>
<td></td>
</tr>
</tbody>
</table>

5.8 servings vs 12 for 14 d. Urinary MDA minimal affected
DNA Strand Breaks

## Low or High VF Diets and Urinary 8-EPG

<table>
<thead>
<tr>
<th>Diet¹</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mg creatinine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low VF¹</td>
<td>4.2 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>-10.9</td>
</tr>
<tr>
<td></td>
<td>(3.9)</td>
<td>(3.81)</td>
<td></td>
</tr>
<tr>
<td>High VF</td>
<td>3.7 ± 0.2</td>
<td>2.5 ± 0.1*</td>
<td>-30.7</td>
</tr>
<tr>
<td></td>
<td>(3.3)</td>
<td>(2.3)</td>
<td></td>
</tr>
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</table>

5.8 vs 12 servings.

Vitamin E

Mitochondrion

tocopherol a free radical (peroxyl) scavenger within membranes

-TH + LOO° → -T° + LOOH

-T° + ascorbate → -TH + ascorbate°
Effects of Dietary Supplements on Oxidative Damage Markers

- In several studies, vitamin E and diet supplement mixtures have been shown to favorably influence indicators of oxidative status, such as susceptibility of LDL to oxidation.

- Randomized clinical trials have generally not confirmed a beneficial effect of supplements (e.g., vitamin E) on disease risk or outcome (Blumberg Nutr Clin Care 2002;5:50-5).
α-tocopheryloxybutyric Acid (TE) and Cell Apoptosis (% Control)

TE = modified vitamin E with no antioxidant properties

Arachidonic Acid Metabolic Pathway

Linoleic Acid

Arachidonic Acid → Ceramide → Apoptosis

Lipoxygenases

HETEs

Leukotrienes

Cyclooxygenases

Dietary Antioxidants

Fatty Acids

Dietary Antioxidants

Prostaglandins (PGG2)

Other Prostaglandins

Thromboxanes

PPARα

PPARδ
ATBC Study
Cancers According to Vitamin E Treatment

- **Lung**: 433 (Vitamin E) vs 443 (No Vitamin E)
- **Prostate**: 99 (Vitamin E) vs 147 (No Vitamin E)
- **Bladder**: 81 (Vitamin E) vs 74 (No Vitamin E)
- **Colorectum**: 66 (Vitamin E) vs 81 (No Vitamin E)
- **Stomach**: 70 (Vitamin E) vs 56 (No Vitamin E)
- **Other**: 378 (Vitamin E) vs 357 (No Vitamin E)

**Number of Cancers**

- Range: 0 to 600

**Legend**

- Yellow: Vitamin E
- Blue: No Vitamin E
Prostate Cancer Prevention Selenium and Vitamin E
Cancer Prevention Trail (SELECT) ($175M)
32,000 men, age $\geq 55$ ($\geq 50$ for Black Men)

- Selenium 200 $\mu$g/d
- Vitamin E 400 mg/d
- Vitamin E Placebo

7 + Years Treatment
Endpoint Prostate Cancer Incidence
The TRAMP Mouse

- Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) animal model that expresses the oncogene SV40 T antigen specifically in the epithelium of the prostate.


![Graph showing progression of prostate cancer in TRAMP model](Greenberg et al. (Found on TRAMP webpage))
Membrane Phospholipids

Arachidonic Acid

PLA₂

TEA

COX-2

PGE₂

TEA

c-Myc

Proliferation

β-Catenin

Tcf

APC

AP-1

Ras

Map Kinases

β-Catenin

Tcf

TEA

PGE₂

TEA

Wargovich
(personal communication)

Tumorigenesis
Candidate Genes Responsive to (-) Epigallocatechin-3-Gallate in Human Prostate Cancer (LNCaP) Cells

**GENES INDUCED**
- Tyrosine receptor kinase type E mRNA
- Phosphoglycerate kinase
- Adenylate kinase 2A
- CDK8 protein kinase
- Putative serine/threonine protein kinase
- Ribosomal protein kinase B
- Mevalonate kinase
- Protein tyrosine phosphatase
- Prostatic acid phosphatase
- Receptor-type protein tyrosine phosphatase γ
- Protein tyrosine phosphatase IC
- STE-20 related kinase SPAK
- IAR/receptor-like protein tyrosine phosphatase
- Pyrroline 5-carboxylate synthase
- Glomerular epithelial protein 1
- Platelet-derived growth factor A type receptor

**GENES REPRESSED**
- Protein kinase C-α
- 41 kDa protein kinase related to rat ERK2
- Type 1b cGMP-dependent protein kinase
- Adenosine kinase short form
- Phosphatidylinositol 3-kinase homolog
- Protein tyrosine phosphatase PIR1
- Protein tyrosine phosphatase zeta
- KIAA0369 gene
- Leukocyte common antigen T20O

Wang and Mukhtar Cancer Lett 2002;182:43
Protein Oxidation

- Proteins are major targets for ROS (Davies et al Free Rad Biol Med 1999)
  - Long half lives, a cumulative indicator
- Measurements:
  - Protein carbonyls: general marker of oxidation in vivo (Stadtman and Berlett Drug Metabol Rev, 1998)
  - Specific Protein Oxidation Products
    - Ease of oxidation of aromatic side-chains
      - Formation of o-Tyr, m-Tyr, di-tyrosine:
    - Protein inactivation (MnSOD)
Protein Oxidation

Amyloid beta-peptide (Abeta) is a 42-43 amino acid peptide known to accumulate in Alzheimer's disease (AD) brain. The neurotoxicity caused by Abeta is a result of its associated free radicals, which can play an important role in generating oxidative stress. Increased protein oxidation, reactive oxygen species (ROS) formation, and neurotoxicity induced by Abeta(1-42) in primary rat embryonic hippocampal neuronal culture are prevented by the free radical scavenger and antioxidant vitamin E.

ROS and Apoptosis

There is a growing body of evidence that ROS may not only regulate apoptotic signal transduction, but also activate apoptotic death pathways.

Vitamin E Inhibits Cisplatin-Induced Apoptosis in MCF-7 Breast Cancer Cells.

Antioxidant Depletion Inhibits Brain Tumor Growth.

One Size Does Not Fit All!
Just when I knew all of life's answers, they changed all the questions.