History of tocopherols
- In 1905, William Fletcher found if special factors were removed from food, disease (Beriberi) occurred
- Vitamin E discovered in 1922 in green leafy vegetables by Herbert Evans and Katherine Bishop
- Because vitamin E supported fertility, it was named tocopherol – Greek tōkos = childbirth, phero = bring forth, al = alcohol
- In 1936, found abundant in wheat germ oil
- In 1938, chemically synthesized
- In nature, eight compounds found to have vitamin E activity: α-, β-, γ- and δ-tocopherol; and α-, β-, γ- and δ-tocotrienol (T-3)

Vitamin E activity and bioavailability
<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Activity, IU/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural α-toco (RRR-)</td>
<td>1.49</td>
</tr>
<tr>
<td>Synthetic α-toco (racemic mix)</td>
<td>1.0</td>
</tr>
<tr>
<td>β-toco</td>
<td>0.6</td>
</tr>
<tr>
<td>γ-toco</td>
<td>0.3</td>
</tr>
<tr>
<td>δ-toco</td>
<td>0.015</td>
</tr>
</tbody>
</table>

- α-tocopherol has the highest bioavailability
- Serum concentration of α-tocopherol controlled by liver, which preferentially resecretes only α-tocopherol via the hepatic α-tocopherol transfer protein (TTP)
- Other forms metabolized and excreted, so their blood and cellular concentrations are much lower, thus less researched

Biosynthesis of tocopherols
- Exclusively by photosynthetic organisms
- Chromanol ring and a 15-carbon tail condensed by homogentisate (HGA) phytyltransferase (HPT)
- Tocotrienols are the primary form in the seed endosperm of monocots (wheat, rice, and barley), rarely in vegetative tissues
- Tocopherols occur ubiquitously in leaves and seeds of dicots
- Transgenic expression of the barley HGGT, which catalyzes the committed step of tocotrienol synthesis, resulted in accumulation of T-3
- Overexpression of the barley HGGT in corn resulted in an increase in total toco by six-fold
**Vitamin E function – as antioxidant**
- Antioxidants protect cells from the damaging effects of free radicals
- Free radicals damage cells, thus contributing to the development of cardiovascular disease and cancer
- Unshared electrons react rapidly with oxygen to form reactive oxygen species (ROS)
- Body forms ROS endogenously and antioxidants might protect cells by inactivating ROS and free radicals

**Vitamin E function – cell signaling, gene and metabolic regulation**
- Members in the vitamin E family possess unique biological functions
- α-Tocopherol may have functions independent of its antioxidant property – it strongly inhibits platelet adhesion
- γ-Tocopherol exhibit functions that are not shared by α-tocopherol, such as the anti-inflammatory effect
- Epidemiological data suggests that γ-tocopherol is a better negative risk factor for certain types of cancer
- Vitamin E-replete endothelial cells at the interior surface of blood vessels resist blood-cell adhering to this surface
- Vitamin E modulates arachidonic acid metabolism – increasing prostacyclin that dilates blood vessels

**General function and mechanism of T-3**
- Tocotrienols have strong neuroprotective, antioxidant, anti-cancer and cholesterol lowering properties that tocopherols do not
- T-3 in μM suppresses HMG-CoA reductase, the enzyme responsible for cholesterol synthesis
- T-3s are thought to be more potent antioxidants than tocos due to their efficient penetration into tissues
- Although the transport T-3 is low, orally supplemented T-3 results in plasma T-3 of 1 μM, a conc of an order of magnitude higher than that required to protect neurons from damage
- Nanomolar α-T-3 prevents neurodegeneration by regulating specific mediators of cell death
- T-3 but not tocopherol, suppresses growth of human breast cancer cells

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Effect on PKC activity is largely on membrane

Vitamin E on coronary heart disease –
(http://ods.od.nih.gov/FACTSHEETS/VITAMINE.ASP)

- Observational studies associated lower rates of heart disease with higher vitamin E intake - 90,000 nurses had 30% to 40% lower heart disease.
- Effects on the heart and blood vessels of healthy women (40,000 ≥45 years of age, 600 IU E for 10 years) - no significant differences in cardiovascular events or mortality
- Randomized clinical trials showed controversial results:
  - The HOPE study (10,000 patients at high risk followed for 4.5 years, 400 IU/day) - no fewer cardiovascular events
  - The HOPE-TOO follow-up study (4,000 participants for another 2.5 years) - no protection against CHD
  - A men's cardiovascular study (15,000 healthy physicians ≥50 years of age with 400 IU for 8 y) - no effect on major cardiovascular events
- Clinical trials have not provided evidence that vitamin E prevents cardiovascular disease. More extensive studies in younger and healthier participants taking higher doses of the supplement is needed

Vitamin E on cancer

- Link between high intake of vitamin E with a decreased incidence of breast and prostate cancers is inconsistent:
- Study on the incidence of breast cancer (18,000 women) - no benefit
- A cohort study (29,000 men) - no association with prostate cancer risk
- A large clinical trial (7-12 years of 400 IU E) – no protection against prostate cancer in healthy men
- An epidemiologic study showed reduced risk of death from bladder cancer
- The inconsistent and limited evidence precludes any recommendations about using vitamin E supplements to prevent cancer

Vitamin E on cognitive function

- Brain has a high oxygen consumption and abundant polyunsaturated fatty acids
- Free-radical can damage neurons thus contributing to cognitive decline and neurodegenerative diseases, such as Alzheimer’s
- Vitamin E’s protection is supported by a clinical trial (341 patients with Alzheimer on 2,000 IU/day E for 2 years) - significantly delayed deterioration
- Vitamin E consumption was associated with less cognitive decline over 3 years of elderly, 65-102 years of age
- A clinical trial in healthy older women (600 IU E for 4 years) - no apparent cognitive benefits
- Another trial (769 with cognitive impairment, 2,000 IU/day E) - no significant differences in Alzheimer rate
- Therefore, most research results do not support the use of vitamin E to maintain cognitive performance

Natural vs synthetic tocos, and health risks from excessive vitamin E

- d-α-Tocopherol (stereoisomeric) vs dl-α-tocopherol (mixture of 8 stereoisomers)
- High doses of α-tocopherol supplements may cause hemorrhage and interrupt blood coagulation in animals
- Increased risk of hemorrhagic stroke in participants taking α-tocopherol
- One meta-analysis of randomized trials found an increased risk of death at doses of ≥400 IU/day
- The implications of these analyses for the potential adverse effects of high-dose vitamin E are unclear – participants middle-aged or older and with chronic diseases. More investigation is needed

Selected food sources of vitamin E (α-tocopherol)

<table>
<thead>
<tr>
<th>Food</th>
<th>Milligrams (mg) /serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ oil, 1 tablespoon</td>
<td>20.3</td>
</tr>
<tr>
<td>Almonds, dry roasted, 1 ounce</td>
<td>7.4</td>
</tr>
<tr>
<td>Sunflower seeds, dry roasted, 1 ounce</td>
<td>6.0</td>
</tr>
<tr>
<td>Hazelnuts, dry roasted, 1 ounce</td>
<td>4.3</td>
</tr>
<tr>
<td>Peanuts, dry roasted, 1 ounce</td>
<td>2.2</td>
</tr>
<tr>
<td>Sunflower oil, 1 tablespoon</td>
<td>5.6</td>
</tr>
<tr>
<td>Corn oil, 1 tablespoon</td>
<td>1.9</td>
</tr>
<tr>
<td>Soybean oil, 1 tablespoon</td>
<td>1.1</td>
</tr>
<tr>
<td>Spinach, boiled, ½ cup</td>
<td>1.9</td>
</tr>
</tbody>
</table>

RDA for vitamin E (alpha-tocopherol) ranges between 4 mg (6 IU) for newborn to 15 mg (22.4 IU) for 14 and older
Tocopherols and T-3 as antioxidants in bulk oil – A comparative study

1. Mechanism of antioxidation is free radical resonance stabilization
2. Antioxidant potency is δ > γ > α

Sources of tocotrienols (T-3)

- Vegetable oils
  - Palm Oil
  - Rice Bran
- Barley
- Oat
- Wheat Germ
- Corn (GMO)

Sources of selected oils

<table>
<thead>
<tr>
<th>Sources</th>
<th>Tocopherols (ppm)</th>
<th>Tocotrienols (T-3, ppm)</th>
<th>Total T-3</th>
<th>Total All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alpha</td>
<td>beta</td>
<td>gamma</td>
<td>delta</td>
</tr>
<tr>
<td>Palm Oil</td>
<td>152</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rice bran</td>
<td>324</td>
<td>18</td>
<td>53</td>
<td>-</td>
</tr>
<tr>
<td>Barley</td>
<td>350</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Oat</td>
<td>180</td>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Wheat Germ</td>
<td>1,179</td>
<td>398</td>
<td>493</td>
<td>118</td>
</tr>
<tr>
<td>Corn</td>
<td>282</td>
<td>54</td>
<td>1,034</td>
<td>54</td>
</tr>
<tr>
<td>Corn Control</td>
<td>420</td>
<td>26</td>
<td>677</td>
<td>25</td>
</tr>
<tr>
<td>Corn HGGT</td>
<td>249</td>
<td>28</td>
<td>607</td>
<td>58</td>
</tr>
</tbody>
</table>

Oxidative stability of corn oil with elevated toco and T-3 - Analysis of oxidation products

- Primary oxidation product
  - Development of hydroperoxides @ 60°C
  - Measured by peroxide value (PV)
- Secondary products
  - Smaller volatile compounds from FA cleavage
  - Measured by conductivity using an OSI Instrument

Model oil system - Toco & T-3 added to purified oil

<table>
<thead>
<tr>
<th>Stripped RBD Corn Oil spiked with:</th>
<th>Concentration (ppm):</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Toco</td>
<td>100</td>
</tr>
<tr>
<td>α-T-3</td>
<td>250</td>
</tr>
<tr>
<td>δ-Toco</td>
<td>700</td>
</tr>
<tr>
<td>δ-T-3</td>
<td>2,000</td>
</tr>
<tr>
<td>γ-T-3</td>
<td>5,000</td>
</tr>
</tbody>
</table>

Peroxide Value of Crude Corn Oil at 60°C

- 2015 Corn
- 2015 HGGT
- 2015 Control
- 2015 HGGT

Day

Δ PV/day 2005 2006
Control 11.96 ± 1.06 9.19 ± 0.34
HGGT 9.83 ± 0.32 8.48 ± 0.27
Peroxide Value (PV) of Corn Oil at 60°C with Alpha Tocotrienol added

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>αToc</th>
<th>αT-3</th>
<th>δToc</th>
<th>δT-3</th>
<th>γT-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm</td>
<td>6.89 ± 0.17</td>
<td>6.73 ± 0.02</td>
<td>5.34 ± 0.08</td>
<td>7.26 ± 0.52</td>
<td>5.43 ± 0.58</td>
</tr>
<tr>
<td>250 ppm</td>
<td>8.87 ± 0.09</td>
<td>8.37 ± 0.70</td>
<td>4.98 ± 0.14</td>
<td>6.88 ± 0.24</td>
<td>5.01 ± 0.33</td>
</tr>
<tr>
<td>700 ppm</td>
<td>15.51 ± 0.10</td>
<td>12.85 ± 0.64</td>
<td>5.25 ± 0.10</td>
<td>6.42 ± 0.46</td>
<td>4.73 ± 0.56</td>
</tr>
<tr>
<td>2,000 ppm</td>
<td>20.68 ± 0.59</td>
<td>25.43 ± 1.02</td>
<td>4.02 ± 0.08</td>
<td>5.48 ± 0.46</td>
<td>7.26 ± 0.43</td>
</tr>
<tr>
<td>5,000 ppm</td>
<td>30.28 ± 0.81</td>
<td>38.33 ± 2.04</td>
<td>4.63 ± 0.05</td>
<td>5.36 ± 0.38</td>
<td>5.25 ± 0.08</td>
</tr>
</tbody>
</table>

Control = Stripped control = 10.09 ± 0.35 APV/day

Dolde and Wang, 2009

Effect of toco and T-3 on oil oxidation

Oil stability index - secondary oxidation products

Conclusions of in vitro antioxidant study in bulk oil

- Antioxidant properties of tocotrienols (T-3) are similar to those of tocopherols in vitro
- At higher concentrations, alpha is a less effective antioxidant than delta and gamma
- All homologues inhibited secondary oxidative products even at very high concentrations (5,000 ppm) but with diminishing effectiveness
- Alpha tocols propagated primary oxidation at concentrations as low as 700 ppm and pro-oxidant effects were increased with higher concentrations
Conclusion remarks

- Tocopherols represent one of the most fascinating natural compounds that have the potential to influence a broad range of human health and disease

- The current state of knowledge warrants study of the lesser known forms of vitamin E, i.e. tocotrienols, that have significant biological functions