Tocopherols and tocotrienols—structure and function

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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 *tokos* = childbirth, *phero* = bring forth, *ol* = 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).
Structure of toco and T-3

A
R1 = R2 = R3 = Me, α-tocopherol
R1 = R3 = Me; R2 = H, β-tocopherol
R1 = H; R2 = R3 = Me, γ-tocopherol
R1 = R2 = H; R3 = Me, δ-tocopherol

B
R1 = R2 = R3 = Me, α-tocotrienol
R1 = R3 = Me; R2 = H, β-tocotrienol
R1 = H; R2 = R3 = Me, γ-tocotrienol
R1 = R2 = H; R3 = Me, δ-tocotrienol
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>
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</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) phytoltransferase (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
Biosynthesis of tocopherols and tocotrienols

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
Activity of T-3 on breast cancer cells

Fig. 29.1. Inhibition of ER–MDA-MB-435 human breast cancer cells by palm oil tocotrienols, as measured by incorporation of tritiated thymidine. Abbreviation: TCR, tocotrienol-rich fraction.

(Guthrie and Carroll, 1998)
Activity of T-3 on breast cancer cells

Fig. 29.2. Inhibition of ER+ MCF-7 human breast cancer cells by palm oil tocotrienols, as measured by incorporation of tritiated thymidine.
Effect of T-3 on PKC

Fig. 29.3. Inhibition of PKC activity in ER- MDA-MB-435 human breast cancer cells by palm oil tocotrienols. Cells were grown to confluency in Petri dishes. The cell culture medium containing fetal calf serum was replaced with medium containing 1% vol/vol bovine serum albumin and the various tocotrienols and incubated overnight. The cells were then treated with phorbol 12-myristate, 13-acetate, to activate PKC. Activity was measured by using a Pierce colorimetric PKC assay kit.
Effect on PKC activity is largely on membrane

**Fig. 29.4.** Inhibition of protein kinase C in the cytosolic and membrane fractions of ER–MDA-MB-435 human breast cancer cells.
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. Finnish (5,133 followed for 14 years) had decreased mortality
- 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 $\delta > \gamma > \alpha$
Sources of tocotrienols (T-3)

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

Source:/www.tocotrienol.org
## Toco concentration 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>-</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

Stripped RBD Corn Oil spiked with:
- αToco
- α T-3
- δ Toco
- δ T-3
- γ T-3

Concentration (ppm):
- 100
- 250
- 700
- 2,000
- 5,000
Peroxide Value of Crude Corn Oil at 60°C

<table>
<thead>
<tr>
<th>ΔPV/day</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.96 ± 1.06</td>
<td>9.19 ± 0.34</td>
</tr>
<tr>
<td>HGGT</td>
<td>9.83 ± 0.32</td>
<td>8.48 ± 0.27</td>
</tr>
</tbody>
</table>
Peroxide Value (PV) of Corn Oil at 60°C with Alpha Tocotrienol added

PV

Day

Control

100 ppm

250 ppm

700 ppm

2,000 ppm

5,000 ppm
Peroxide Value of Corn Oil at 60°C with Alpha Tocopherol Added
Effect of toco and T-3 on oil oxidation

Change of PV/day of corn oil with added tocols at 60°C in the dark

<table>
<thead>
<tr>
<th>ΔPV/day</th>
<th>α Toco</th>
<th>αT-3</th>
<th>δ Toco</th>
<th>δ T-3</th>
<th>γ T-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>13.51 ± 0.10</td>
<td>12.85 ± 0.64</td>
<td>5.21 ± 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>38.35 ± 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 ΔPV/day

Dolde and Wang, 2009
Oil stability index - secondary oxidation products
OSI - induction time @ 100°C

OSI Induction Period Amount/Hours

Graph showing the induction period and amount in hours for different concentrations of AT3, Alpha Toco, DT3, Delta Toco, and GT3.
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.