Carotenoids, Health Benefits and Bioavailability

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Phytochemicals in Fruits and Vegetables for Health
October 2, 2013

Carotenoids

- Phytoene
- Phytofluene
- \( \beta \)-Carotene
- Neurosporene
- Lycopene

Lycopene Biosynthesis

- Phytoene
- Phytofluene
- \( \xi \)-Carotene
- Neurosporene
- Lycopene

Biosynthesis of common \( \beta \) and \( \varepsilon \) cyclic carotenes

- Lycopene
- \( \beta \)-Carotene
- \( \gamma \)-Carotene
- \( \alpha \)-Carotene
- \( \varepsilon \)-Carotene

Adapted from Belton, 1993 and Gross, 1992.

Tomatoes Varieties with Unique Carotenoid Profile

RED OR1 OR2 YEL GRE
**Common Carotenoids**

Xanthophylls

- Lutein
- Zeaxanthin
- β-Cryptoxanthin

Hydrocarbons

- α-Carotene
- β-Carotene
- Lycopene

**Biological Functions of Carotenoids**

- **Provitamin A Activity**
- **Non-provitamin A Activity:**
  - Singlet Oxygen Quenching Activity
  - Antioxidant Activity (Trap Free Radicals)
  - Enhancement of Immune Response
  - Potential Chemopreventive Properties

**Conversion to Vitamin A**

\[
\text{β-carotene} \xrightarrow{\text{O}_2} 15,15'-\text{oxygenase} \xrightarrow{\text{H}^+} \text{reductase} \rightarrow \text{retinol}
\]

**Vitamin A - WHO Facts and Figures**

- An estimated 250 million preschool children are vitamin A deficient
  - It is likely that in vitamin A deficient areas, a substantial proportion of pregnant women are vitamin A deficient
- An estimated 250 000 to 500 000 vitamin A-deficient children become blind every year,
  - Half of these die within 12 months of losing their sight

**Carotenoids and Health Benefits**

- Epidemiological
- Cell culture
- Animal (experimental)
- Human (clinical)
Dietary carotenoids, vitamin-A, vitamin-C, and vitamin-E, and advanced Age-related Macular Degeneration

Seddon et al.

“Conclusion.-Increasing the consumption of foods rich in certain carotenoids, in particular dark green, leafy vegetables, may decrease the risk of developing advanced or exudative AMD, the most visually disabling form of macular degeneration among older people.”

Lutein & Zeaxanthin in the Macula

- Macula is the Region Directly Behind the Lens, Receiving the Most Light.
- Lutein & Zeaxanthin are Deposited Specifically.
- The Role Is To Prevent Photo-Oxidation.

Vegetables, fruit, and cancer prevention: A review

Steinmetz KA, Potter JD
Journal of the American Dietetic Association 96: (10) 1027-1039, 1996

“The types of vegetables or fruit that most often appear to be protective against cancer are raw vegetables, followed by allium vegetables, carrots, green vegetables, cruciferous vegetables, and tomatoes.”

Absorption and Bioavailability

Bioavailability is the fraction of an ingested nutrient that is available for utilization in normal physiological functions, metabolism and/or storage.

In the context of this discussion, bioavailability of carotenoids is defined as the amount of these micronutrients that are absorbed by the intestinal enterocytes and transported in the bloodstream.
**Food Processing and Preparation**

- Physical and thermal treatment of foods causes degradation of plant cell structural constituents.
- Thermal processing brings about disruption of the carotenoid protein complexes.
- Inactivation of oxidizing enzymes results in less degradation and greater stability of carotenoids.
- These changes result in enhanced uptake and efficiency of carotenoid absorption.

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**Intake of carotenoids and retinol in relation to risk of prostate cancer**

Giovannucci et al.
*Journal of the National Cancer Institute, 87(23): 1767-76, 1995*

“Combined intake of tomatoes, tomato sauce, and pizza (which accounted for 82% of lycopene intake) was inversely associated with risk of prostate cancer.”

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**Circulating Carotenoids and Risk of Breast Cancer: Pooled Analysis of Eight Prospective Studies**

A. Heather Eliassen et al.
*Journal of the National Cancer Institute, 104:1905–1916, 2012*

“This comprehensive prospective analysis suggests women with higher circulating levels of a-carotene, β-carotene, lutein/zeaxanthin, lycopene, and total carotenoids may be at reduced risk of breast cancer.”

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**CARET and ATBC (randomized, double blind, placebo controlled)**

- Supplemented with high dose of beta-carotene (20mg and 30mg)
- Followed for 5-8 years
- Risk of lung cancer increased in both studies in smokers


Carotenoids as Antioxidants

Endogenous and Exogenous Reactive Oxygen Species (ROS)

Lipids, Proteins, DNA Damage

Chronic Disease

Lycopene as a Singlet Oxygen Quencher

- Lycopene is the most efficient singlet oxygen quencher and phenoxyl radical scavenger among the naturally occurring carotenoids.


Other Hypothesized Mechanism of Action

- Stimulate enzymes that detoxify toxins/carcinogens
- Enhance DNA repair mechanisms
- Increase apoptosis of cancer cells
- Decrease cell proliferation
- Possess anti-angiogenesis activity
- Restore gap-junction communication

PDA Chromatogram of Tomato Carotenoids

Selected Geometrical Isomers of Lycopene

- all-trans lycopene
- 5-cis lycopene
- 9-cis lycopene
- 13-cis lycopene
- 7,9,9',7'-cis lycopene
Lycopene is more bioavailable from tomato paste than from fresh tomatoes

Christine Gärtner, Wilhelm Stahl and Helmut Sies

ABSTRACT Lycopene bioavailability, from a single dose of fresh tomatoes or tomato paste (23 mg lycopene) ingested together with 15 g corn oil was compared by analyzing carotenoid concentrations in the chylomicron fraction. The lycopene isomer pattern was the same in both fresh tomatoes and tomato paste. The triacylglycerol response in chylomicrons was not significantly different after both treatments. Ingestion of tomato paste was found to yield 2.5-fold higher total and all-trans-lycopene peak concentrations ($P<0.05$ and $P<0.005$, respectively) and 3.8-fold higher area under the curve (AUC) responses ($P<0.001$) than ingestion of fresh tomatoes. The same was calculated for lycopene cis-isomers, but only the AUC response for the cis-isomers was significantly higher than ingestion of tomato paste ($P<0.005$). No difference was observed in the α- and β-carotene response. Thus, in humans, the bioavailability of lycopene is greater from tomato paste than from fresh tomatoes.

Electron Microscopy

Electron micrograph of lycopene crystalloids in mature red tomato chromoplast.

A. Lycopene crystalloids.
B. Plastoglobulin-type sacs in which β-carotene reportedly accumulates.

Tomato Product Consumption – Correlation with Plasma Lycopene

<table>
<thead>
<tr>
<th>Product</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato Sauce</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>$&lt;0.056$</td>
</tr>
</tbody>
</table>

Lower Prostate Cancer Risk in Men with Elevated Plasma Lycopene Levels: Results of a Prospective Analysis (Gann et al., Cancer Research, 1999, 59:1225-1230)
**Commercial Products Study – Experimental Design**

- **Vegetable Juice**
- **Tomato Sauce**
- **Tomato Soup**

36 subjects
18 M, 18 F

**Wash-out** (14 days)
**Meals** (28 days)
**Analysis**

**Plasma Lycopene Response**

**Total Lycopene Levels in Buccal Cells**

**Clinical Study Design – Prostate Cancer Subjects**

**Change in Plasma Lycopene Concentrations Following 21 Days of Dietary Intervention with Tomato-Based Products or Soy**

**Prostate Lycopene Isomers Following 3 Weeks of Dietary Intervention with Tomato Products or Soy Protein**

1P < 0.05; N = 8/group

1P < 0.01; N = 8/group; Other prostate carotenoids were not sig. across groups
Absorption of \(\beta\)-Carotene and Vitamin A with Different Levels of Fat (adapted from Roels et al. *J. Nutr.* 1958)

<table>
<thead>
<tr>
<th>day</th>
<th>(\beta)-carotene ((\mu\g/100\ ml\ serum))</th>
<th>Vitamin A ((\mu\g/100\ ml\ serum))</th>
<th>(\beta)-carotene ((\mu\g/100\ ml\ serum))</th>
<th>Vitamin A ((\mu\g/100\ ml\ serum))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43.0</td>
<td>36.6</td>
<td>47.8</td>
<td>31.0</td>
</tr>
<tr>
<td>31</td>
<td>80.6</td>
<td>51.0</td>
<td>297.8</td>
<td>50.4</td>
</tr>
<tr>
<td></td>
<td><strong>difference</strong></td>
<td></td>
<td><strong>250.0</strong></td>
<td><strong>19.4</strong></td>
</tr>
</tbody>
</table>

**Massive doses of a carotene-rich vegetable to the basic diet over a long period did not greatly increase serum carotene and vitamin A levels and that most carotene was excreted.**

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**Caco-2 Human Intestinal Cells**

- Human colon carcinoma cells
- Differentiate spontaneously at confluence under normal cell culture condition
- Enterocyte: polarized monolayer, microvilli, tight junctions

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**Caco-2 Cells**

**Uptake**

- Test Media
- DMEM
- Micellar Fraction

**Transport**

- Apical
- Basolateral

**Harvest Cells**

- Collect Basolateral Media
- Analyze for Pigments

**Test Media**

- DMEM
- Micellar Fraction

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**HPLC-MS/MS Analysis**

**Prostate Isoflavones**

**Prostate beta-carotene and lycopene**

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**Two Stage *In Vitro* Digestion**

**Gastric Phase**

- Test Meal
- Gastric Digesta

**Intestinal Phase**

- Intestinal Digesta
- Centrifugation

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**Carotene Balances on Boys in Rwanda Where Vitamin A Deficiency is Prevalent**

Roels, Trout and Dujacquier. *J. Nutr.* 1958

*Massive doses of a carotene-rich vegetable to the basic diet over a long period did not greatly increase serum carotene and vitamin A levels and that most carotene was excreted.*

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Addition of fats to the diet may contribute to the relief of vitamin A deficiency in this region. Roels, Trout and Dujacquier. *J. Nutr.* 1958

Carotenoid Absorption & Metabolism


Post-prandial Response

440 mV Applied Potential 20.0 nA Full Scale

Carotenoid Response at Three Different Lipid Levels


Avocado as Source of Dietary Lipid

Baseline Corrected Mean AUC Values for Each Treatment

Values are based upon mean AUC ± SEM
High β-Carotene Tomatoes

- Monitor absorption of carotenoids from a novel high β-carotene tomato sauce when co-consumed with and without lipid (avocado as source of fat).
- Quantify bioconversion of provitamin A β-carotene as influenced by the presence of lipid in the diet.

LCMS Analysis of β-Carotene and Retinyl Esters

*Modified method also analyzes for α-tocopherol, phytoquinone, lutein, β-cryptoxanthin, α-carotene, lycopene

Delivery of β-Carotene and Retinyl Esters (Vitamin A)

Unilever’s Wish-Bone Salad Dressing

Baseline-corrected carotenoid level in Blood Plasma (nmol/L ± SEM)

β-Carotene
Retinyl Palmitate

Delivery of E-Carotene and Retinyl Esters (Vitamin A)

Sauce + Avocado
Sauce only

Kopec et al. (2013)

Unilever’s Wish-Bone Salad Dressing

Bioactive Metabolites – Retinoids

β-carotene → Lycopene → Retinaldehyde → Lycopenaldehyde → Retinoic Acid → RAR Activation → Transcription

Metabolism of Carotenoids

Bioactive Metabolites – Retinoids

Metabolites - Lycopenals

Lycopenals – LC-MS

Kopec et al. (2013)
Apo-Lycopenals in Human Plasma

Standards
- Lycopene
- apo-6'-lycopenal
- apo-8'-lycopenal
- apo-10'-lycopenal
- apo-12'-lycopenal
- apo-14'-lycopenal

Human Plasma

\[ \text{Kopec et al. (2010)} \]
\[ \text{J. Agric Food Chem.} \]

13C-Lycopene Production using Tomato Cell Cultures

- Tomato flower
- Tomato callus culture (13C-Glucose)
- Tomato cell suspension


13C Lycopene Plasma Response After Dosing

Metabolomics (MS & 13C NMR)

Mass Spectrum of Lycopene Isotopomers in Human Plasma after Single Dose of 13C Lycopene Showing Native and 13C Enriched Forms

13C NMR Spectra of Urine

- Baseline
- Urea
- Creatinine

Post-Dose

β-apo-Carotenals in Human Plasma
Retinoic acid (red) and β-apo-13-carotenone “docked” into the ligand-binding site of RARβ


**Summary**

- Accumulating evidence continues to associate health benefits with carotenoid rich fruit and vegetable consumption.
- “Bioavailability” of carotenoids from specific food products is influenced by a number of factors including food processing treatments, matrix and product formulation (particulary lipid content) and should be determined to more accurately assess delivery of these phytochemicals from the diet.
- Unique varieties of vegetables and fruit (high lycopene, tangerine, high beta carotene tomatoes) can be considered to study the bioavailability and bioconversion of carotenoids from the diet.
- Apo-carotenoid metabolic oxidation products may exhibit biological activity by binding to retinoid receptors and modulate gene expression.

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