

# Carotenoids, Health Benefits and Bioavailability

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Food Science & Interdisciplinary Graduate Program in Nutrition  
The Ohio State University

Phytochemicals in Fruits and Vegetables for Health  
October 2, 2013

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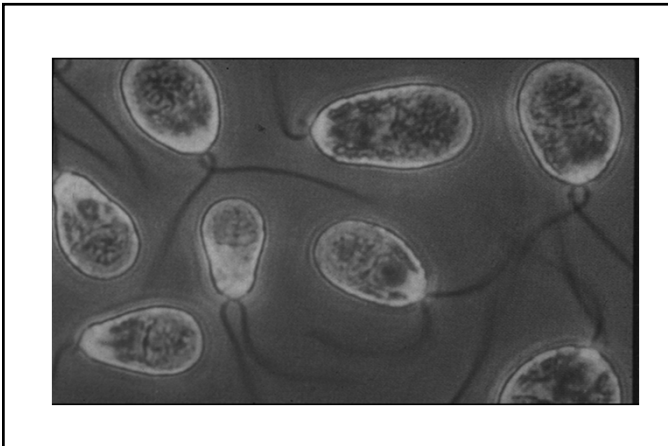
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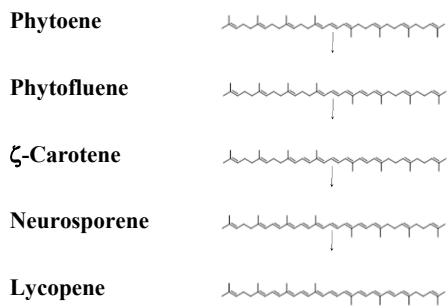
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## Lycopene Biosynthesis




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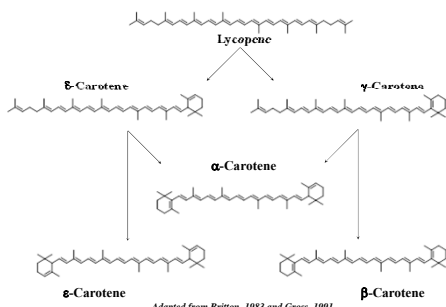
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## Biosynthesis of common β and ε cyclic carotenes




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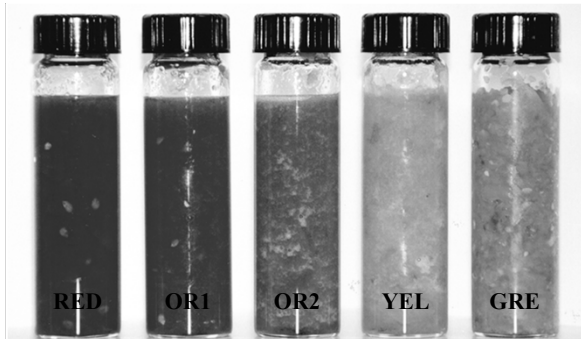
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## Tomatoes Varieties with Unique Carotenoid Profile




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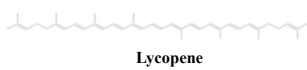
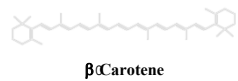
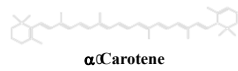
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## Common Carotenoids

### Xanthophylls



### Hydrocarbons



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## 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

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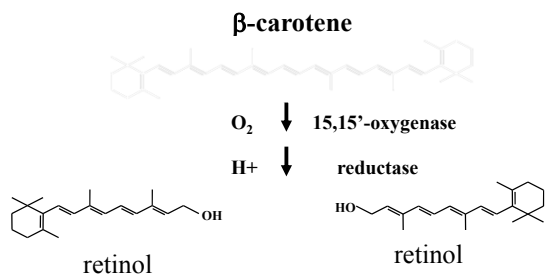
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## Conversion to Vitamin A



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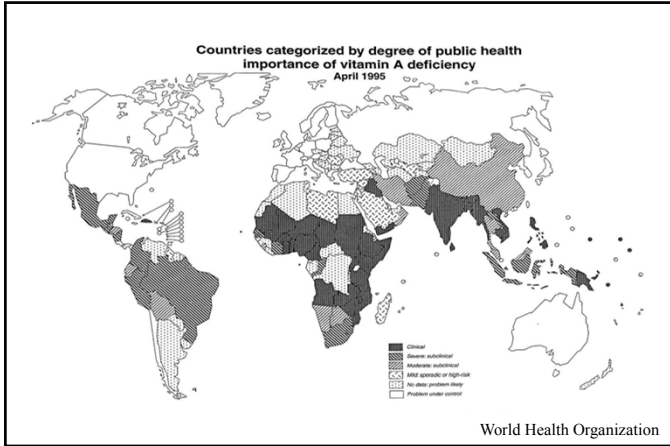
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### Vitamin A - WHO Facts and Figures

- 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

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### Carotenoids and Health Benefits

- **Epidemiological**
- **Cell culture**
- **Animal (experimental)**
- **Human (clinical)**

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## Dietary carotenoids, vitamin-A, vitamin-C, and vitamin-E, and advanced Age-related Macular Degeneration

Seddon et al.  
J. Am. Med. Assoc. 272: (18) 1413-1420, 1994

*“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.”*

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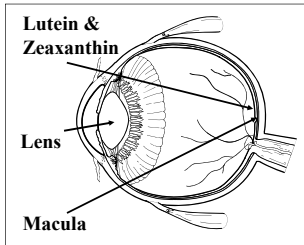
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## Lutein & Zeaxanthin in the Macula

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



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REVIEW ARTICLE

Can dietary beta-carotene materially reduce human cancer rates?

R. Peto\*, R. Doll†, J. D. Buckley‡ & M. B. Sporn§

\* Imperial Cancer Research Fund Cancer Studies Unit, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, UK
† Imperial Cancer Research Fund Cancer Epidemiology Unit, 9 Keble Road, Oxford OX1 3QG, UK
‡ Department of Biomathematics, University of Oxford, Oxford OX1 2JZ, UK
§ Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20205, USA

Human cancer risks are inversely correlated with (a) blood retinol and (b) dietary beta-carotene. Although retinol in the blood might well be truly protective, this would be of little immediate value without discovery of the important external determinants of blood retinol which (in developed countries) do not include dietary retinol or beta-carotene. If dietary beta-carotene is truly protective—which could be tested by controlled trials—there are a number of theoretical mechanisms whereby it might act, some of which do not directly involve its 'provitamin A' activity.

THE possibility of discovering anticancer substances that can be prescribed rather than carcinogens that must be proscribed is attractive, for people may be more willing to accept prescription than proscription. If inhibitors of the late 'stages' of neoplastic progression suggest that the possible relevance of these two components of dietary vitamin A' to cancer prevention should be assessed separately. (2) The inhibition by retinol and various synthetic retinoids of

Horizontal lines for notes or comments.

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."

Horizontal lines for notes or comments.

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.

Horizontal lines for notes or comments.

## 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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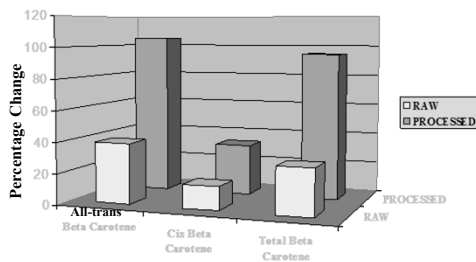
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## Altered “Bioavailability” By Food Processing



Rock, C., Lovalvo, J., Emenhiser, C., Ruffin, M., Flatt, S., Schwartz, S., *J. Nutr.*, 128: 913-916, 1998.

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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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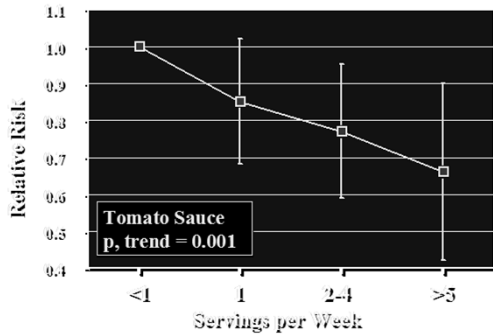
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**Relative Risk of Prostate Cancer (Non A1) and Intake of Tomato Sauce in the HPFS.**




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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  $\alpha$ -carotene,  $\beta$ -carotene, lutein+zeaxanthin, lycopene, and total carotenoids may be at reduced risk of breast cancer.”

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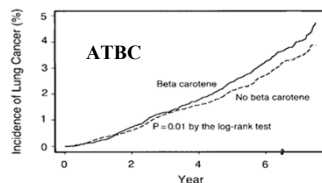
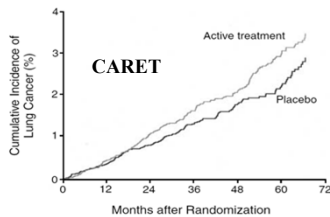
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**CARET and ATBC**

(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

Omenn et al. 1996. New Eng J Med 334(18): 1150-5  
 The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. 1994. New Eng J Med 330(15):1029-35.




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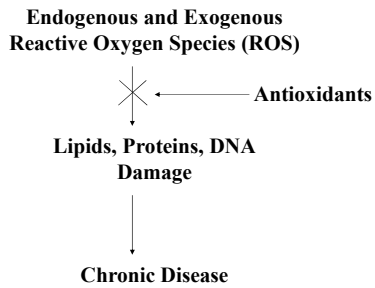
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## Carotenoids as Antioxidants

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## Lycopene as a Singlet Oxygen Quencher

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

Stahl and Sies, 1996. Lycopene: a biologically important carotenoid for humans? *Arch. Biochem. Biophys.* 336:1-9.

Mortensen and Skibsted, 1997. Importance of carotenoid structure in radical-scavenging reactions. *J. Agric. Food Chem.* 45:2970-2977.

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## Other Hypothesized Mechanism of Action

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- 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

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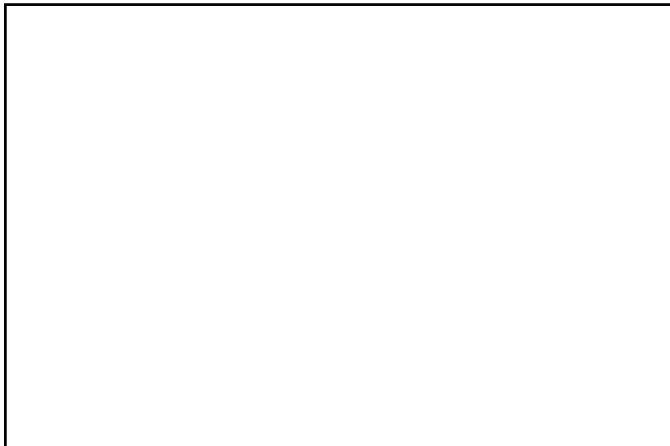
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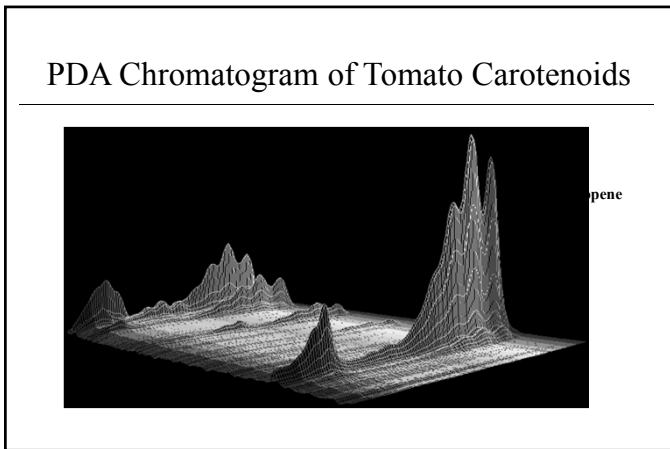
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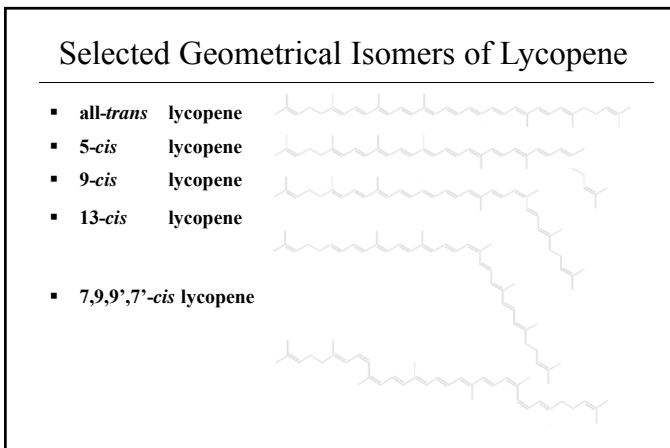
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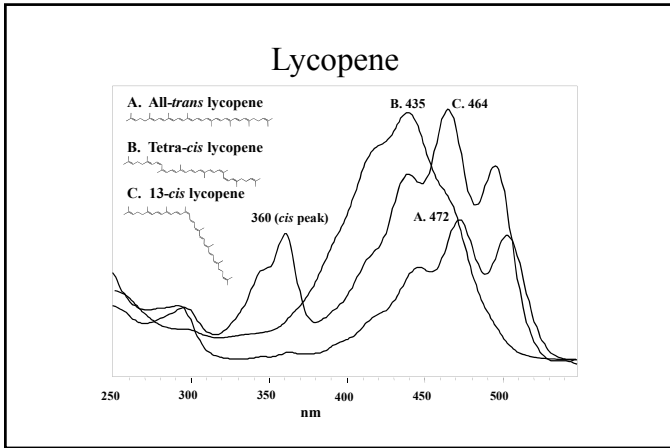
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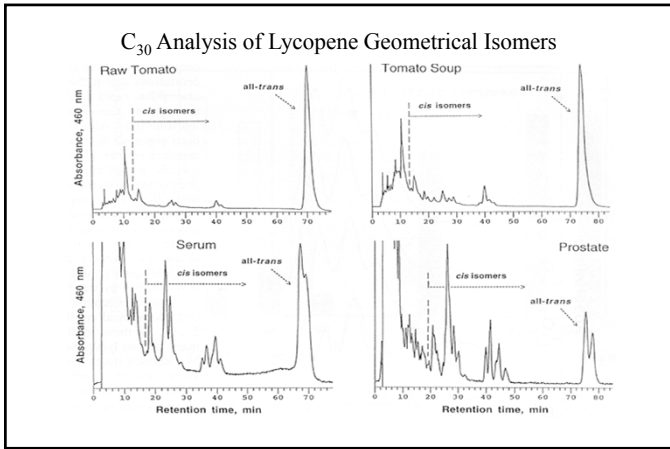
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*Am J Clin Nutr* 1997; 66:116-22

## Lycopene is more bioavailable from tomato paste than from fresh tomatoes

*Christine Gärtner, Wilhem 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  $\alpha$ - and  $\beta$ -carotene response. Thus, in humans, the bioavailability of lycopene is greater from tomato paste than from fresh tomatoes.

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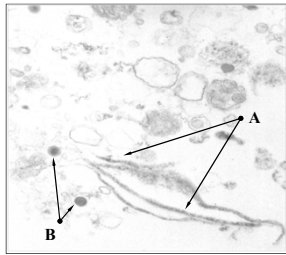
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## Electron Microscopy



**Electron micrograph of lycopene crystalloids in mature red tomato chromoplast.**

A. Lycopene crystalloids.  
B. Plastoglobulin-type sacs in which  $\beta$ -carotene reportedly accumulates.

3.5  $\mu\text{m}$

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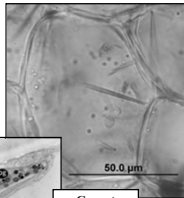
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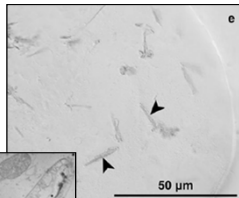
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## Crystalloid Carotenoids in Tomato and Carrot



Carrot root



Tomato fruit

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## Tomato Product Consumption – Correlation with Plasma Lycopene

**Tomato Sauce**                      **p < 0.0001**

**Tomatoes**                              **p < 0.056**

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)

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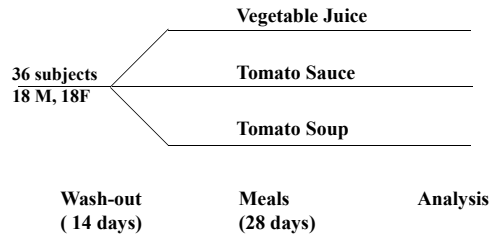
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## Commercial Products Study – Experimental Design




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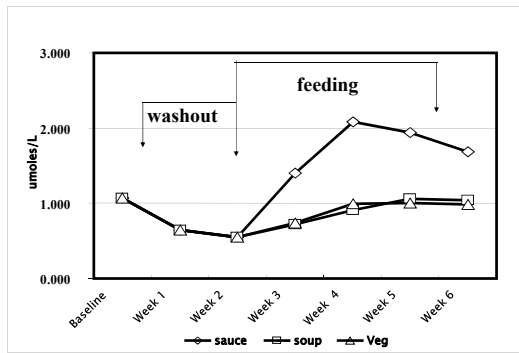
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## Plasma Lycopene Response



Allen et al. J. Nutr. 2003

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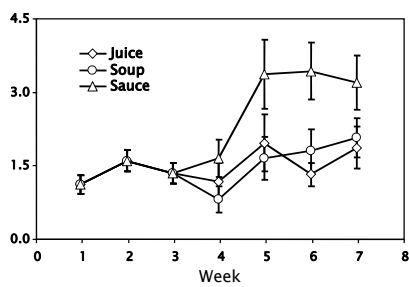
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## Total Lycopene Levels in Buccal Cells



Allen et al. J. Nutr. 2003

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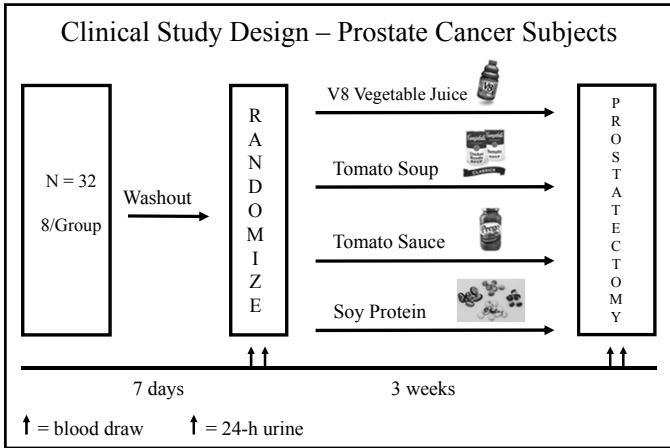
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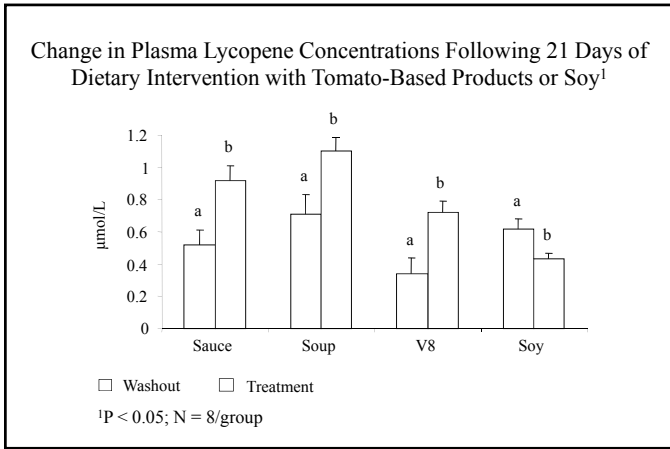
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### Prostate Lycopene Isomers Following 3 Weeks of Dietary Intervention with Tomato Products or Soy Protein<sup>1</sup>

	all- <i>trans</i>	Total <i>cis</i>	Total
	nmol/g		
Tomato Sauce	0.12±0.02 <sup>a</sup>	0.48±0.08 <sup>a</sup>	0.60±0.10 <sup>a</sup>
Tomato Soup	0.11±0.01 <sup>a</sup>	0.46±0.05 <sup>a</sup>	0.58±0.06 <sup>a</sup>
V8 juice	0.07±0.01 <sup>b</sup>	0.30±0.06 <sup>b</sup>	0.37±0.07 <sup>b</sup>
Soy Protein	0.04±0.01 <sup>b</sup>	0.13±0.02 <sup>c</sup>	0.16±0.02 <sup>c</sup>

<sup>1</sup>P < 0.01; N = 8/group; Other prostate carotenoids were not sig. across groups

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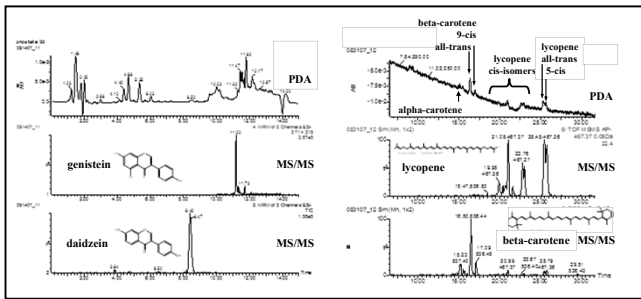
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## HPLC-MS/MS Analysis

### Prostate Isoflavones

### Prostate beta-carotene and lycopene




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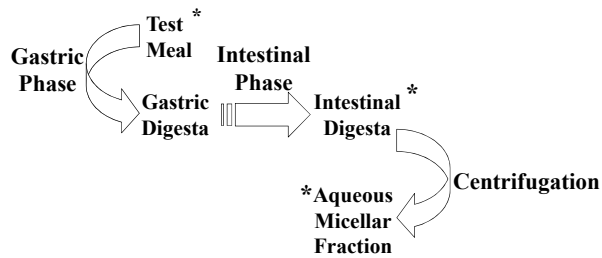
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## Two Stage *In Vitro* Digestion



Garrett et al. (1999) *J. Agric. Food Chem.* 47:4301

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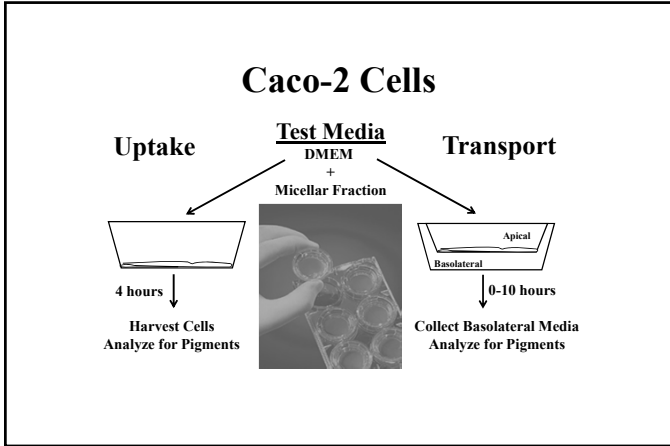
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### Carotene Balances on Boys in Rwanda Where Vitamin A Deficiency is Prevalent

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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### Absorption of $\beta$ -Carotene and Vitamin A with Different Levels of Fat (adapted from Roels et al. *J. Nutr.* 1958)

day	200 g carrots (n=5)		200 g carrots + 20 ml olive oil (n=5)	
	$\beta$ -carotene ( $\mu\text{g}/100\text{ ml serum}$ )	Vitamin A ( $\mu\text{g}/100\text{ ml serum}$ )	$\beta$ -carotene ( $\mu\text{g}/100\text{ ml serum}$ )	Vitamin A ( $\mu\text{g}/100\text{ ml serum}$ )
0	43.0	36.6	47.8	31.0
31	80.6	51.0	297.8	50.4
difference	<b>37.6</b>	<b>14.4</b>	<b>250.0*</b>	<b>19.4</b>

\* significantly different from  $\beta$ -carotene serum levels without fat addition (unpaired Student t-test,  $p < 0.005$ )

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

*Addition of fats to the diet may contribute to the relief of vitamin A deficiency in this region.*

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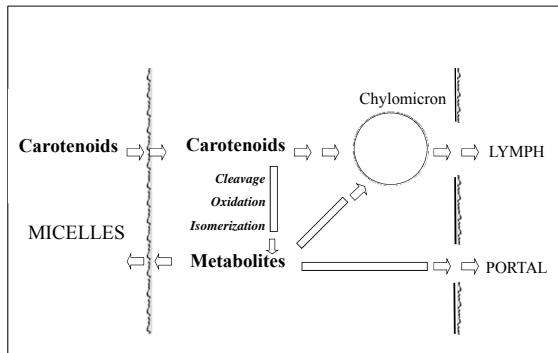
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### Carotenoid Absorption & Metabolism



Adapted from Erdman et al. *Ann. N.Y. Acad. Sci.* 691:76-85 (1993)

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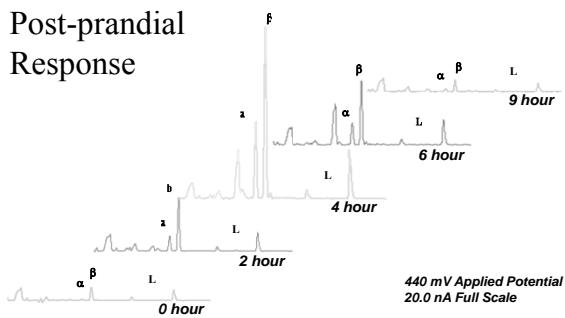
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### Post-prandial Response



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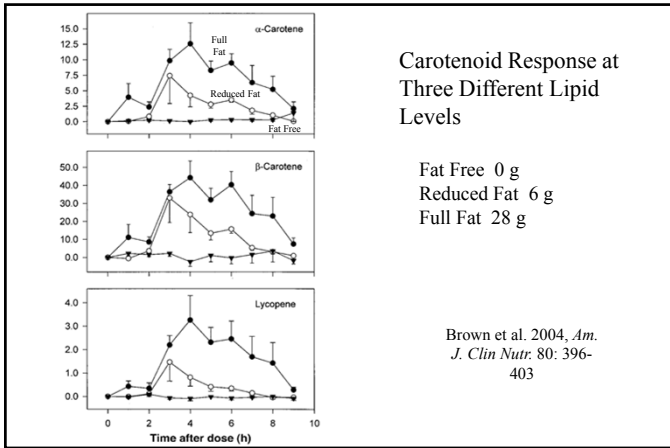
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### Avocado as Source of Dietary Lipid

40g Lettuce    100g Carrots    80g Spinach  
 +  
 75g Avocado / 150g Avocado / 24g Avocado Oil

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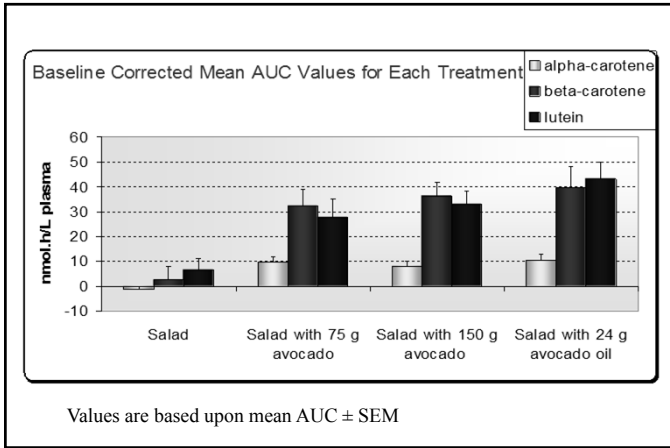
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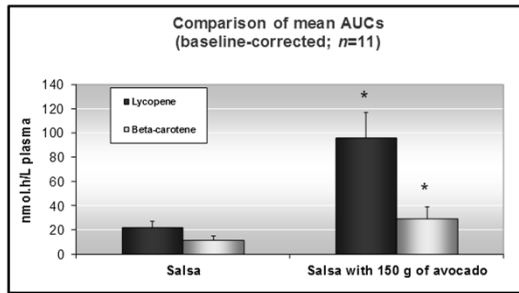
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### Post-prandial Response of Lycopene and Beta-Carotene - Salsa With and Without Avocado



Unlu et al. (2005) *J. Nutr.* 135:431-436

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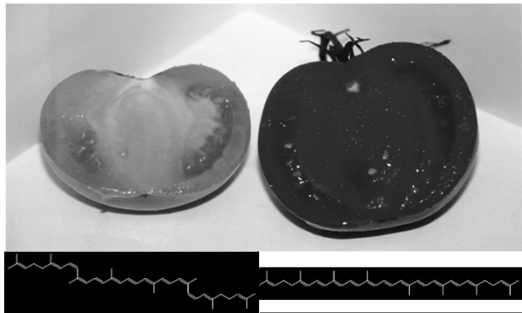
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### Tomato Varieties




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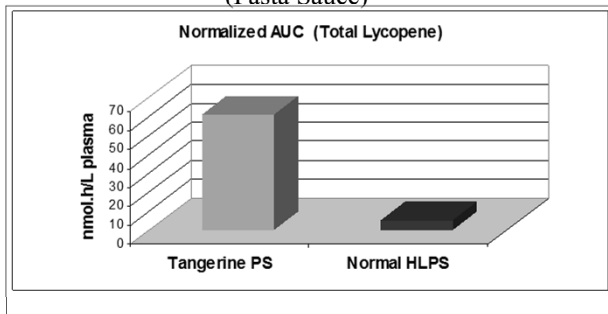
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### Postprandial Absorption of Tangerine vs High Lycopene (Pasta Sauce)




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## High $\beta$ -Carotene Tomatoes

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



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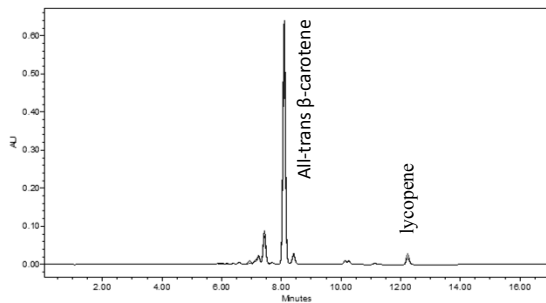
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## High $\beta$ -Carotene Tomato Sauce



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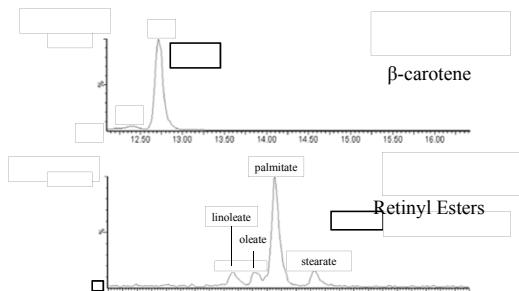
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## LCMS Analysis of $\beta$ -Carotene and Retinyl Esters



\*Modified method also analyzes for  $\alpha$ -tocopherol, phyloquinone, lutein,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene, lycopene

Kopec et al. *Rapid Comm. Mass Spec.* (2013) Fleshman et al., *J. Lipid Res.* (2012)

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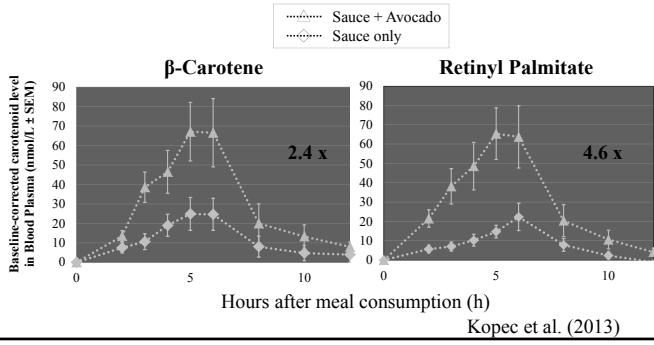
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### Delivery of $\beta$ -Carotene and Retinyl Esters (Vitamin A)




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### Unilever's Wish-Bone Salad Dressing




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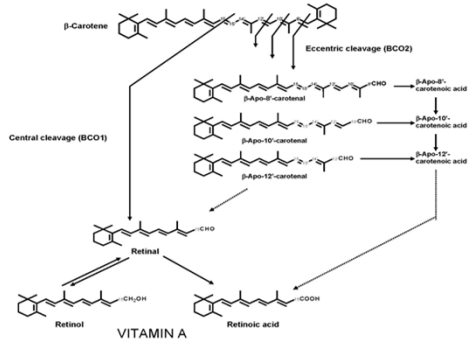
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### Metabolism of Carotenoids




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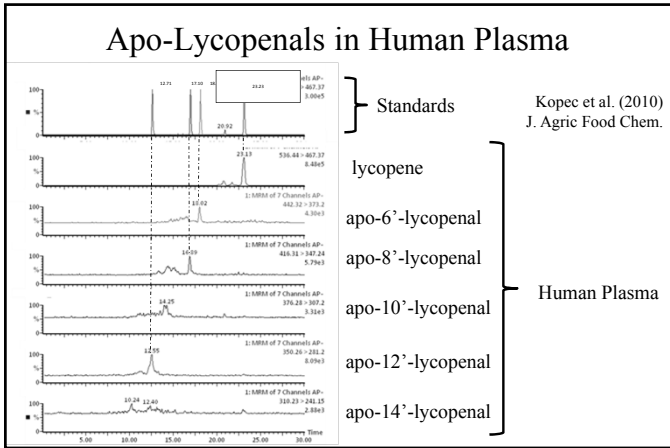
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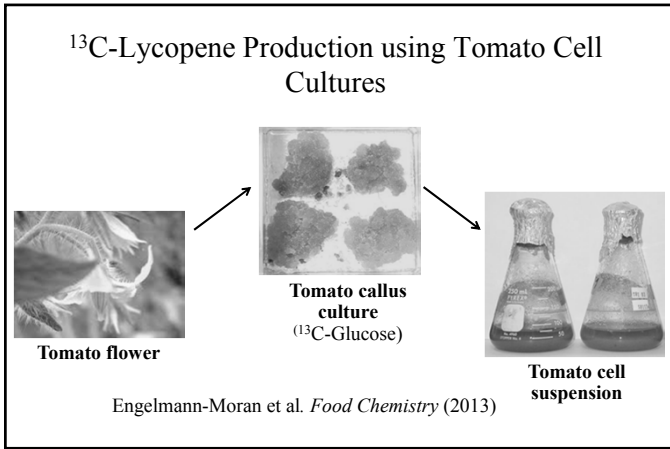
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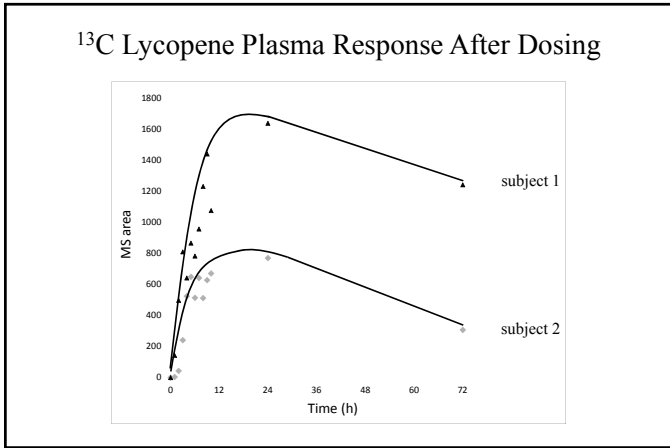
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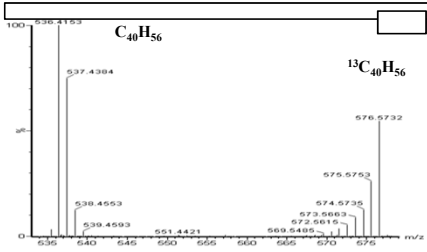
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## Metabolomics (MS & <sup>13</sup>C NMR)

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




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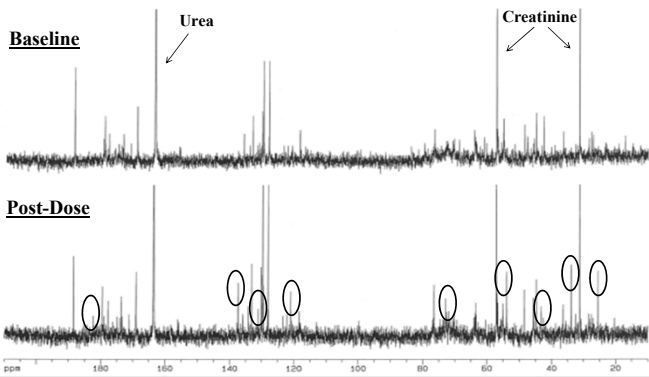
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## <sup>13</sup>C NMR Spectra of Urine




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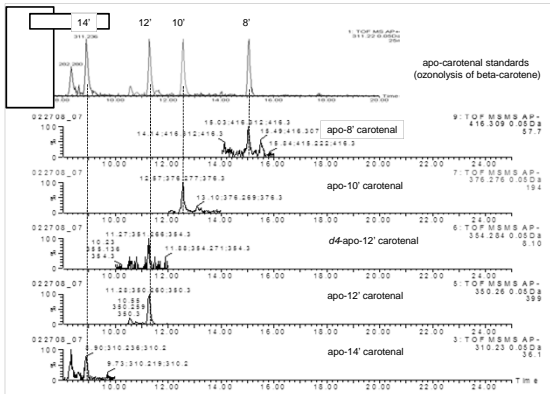
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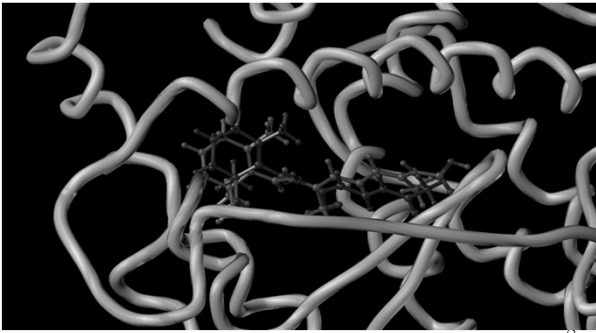
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## β-apo-Carotenals in Human Plasma



Retinoic acid (red) and  $\beta$ -apo-13-carotenone “docked” into the ligand-binding site of RAR $\beta$



Eroglu et al. *J. Biol. Chem* (2012)

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## 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 (particularly 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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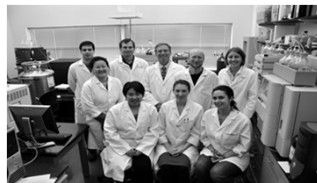
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- Minhthy Nguyen, Ph.D.
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- Torsten Bohn, Ph.D.
- Craig Hadley, Ph.D.
- Robin Ralston, MS



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