Commercial open pond cultivation of *Dunaliella* in the D-Factory

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Brussels

October 17-18, 2017
Synthetic all-trans $\beta$-Carotene

$C_{40}H_{56}$, Molecular Weight 536.85, C 89.49%, H 10.51%
Scheme of Carotenoids Chemical Synthesis, Roche & BASF since~1950

Fig. 16. Chemical schemes of carotenoid synthesis. (Courtesy of J. Surmatis.)
Synthetic all-trans-β-Carotene

Crystal Powder

Formulation to oil emulsion
AT β-Carotene Colored Food
Colored Fish

Synthetic Carotenoids
Colored Eggs

Synthetic Carotenoids
Vitamins, the Boom 1980-1990
“β-Carotene Prevents or Delays Cancer”, Roche 1986

“A capsule a day will keep the cancer away”

Nutritionists have found that people with a daily intake of fruit and vegetables rich in Beta-Carotene have a lower risk of cancer.

Scientists have shown in the laboratory that Beta-Carotene is able to prevent or delay cancer.

Beta-Carotene is a very promising nutritional factor in cancer prevention.

For scientific publications, please contact ROCHE.
β-Carotene
Friend? 1980-90
or
Foe? 1990-2000
The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a cancer prevention trial conducted by the U.S. National Cancer Institute (NCI) and the National Institute for Health and Welfare of Finland from 1985 to 1993. The purpose of the study was to determine whether certain vitamin supplements would prevent lung cancer and other cancers in a group of 29,133 male smokers in Finland. The 50- to 69-year-old participants took a pill daily for five to eight years that contained one of the following: 50 milligrams (mg) alpha-tocopherol (a form of vitamin E), as dl-alpha-tocopheryl acetate, 20 mg of beta-carotene (a precursor of vitamin A), as all-trans-beta-carotene, both, or a placebo (inactive pill that looked like the vitamin).

The participants stopped taking the vitamin supplements in April 1993. However, in order to evaluate the long-term effects of the vitamins on cancer incidence, and overall and cause-specific mortality, they were followed after the trial ended using data from the national registries in Finland. The researchers acquired additional data for cancer incidence and mortality related to specific causes through December 2012 and for total mortality through December 2013 (twenty years beyond the end of the trial).

For more information regarding study design and initial findings, download The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance and the The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers.
Information for CARET Participants

The CARET Repository has been a valuable resource for the continuation of cancer research. To enhance our database, in 2015 we obtained cancer and mortality data from national death indices and the state cancer registries of Washington, California, and Connecticut. We remain committed to maintaining our participants’ confidentiality, and all new data received are secured in the CARET central database. Any data shared with investigators for research will have all identifiers removed to maintain confidentiality. Should you wish to learn more about this, please click to contact CARET staff.

Our Mission

To share biological specimens from the CARET Biorepository with researchers of hypothesis-driven studies investigating cancer causation, early detection, and treatment.

Introduction

The Carotene and Retinol Efficacy Trial (CARET) was a randomized, double-blind, placebo-controlled trial of the cancer prevention efficacy and safety of a daily combination of 30 mg of beta-carotene and 25,000 IU of retinyl palmitate in 18,314 persons at high risk for lung cancer. CARET began in 1985, and the intervention was halted in January 1996, 21 months ahead of schedule, with the twin conclusions for definitive evidence of no benefit and substantial evidence of a harmful effect of the intervention on both lung cancer incidence and total mortality. CARET continued to actively follow and collect endpoints on their participants through 2005. Passive follow-up to extend cancer and death outcomes through 2013 was conducted via linkages with select cancer registries and the National Death Index (NDI). During the active intervention phase of CARET, serum, plasma, whole blood, and lung tissue specimens were collected on participants. These biospecimens make up the CARET Biorepository.
PHYSICIANS' HEALTH STUDY (PHS)

A randomized, double-blind, placebo controlled trial designed to test the effects of low-dose aspirin and beta-carotene in the primary prevention of CVD and cancer among 22,071 US male physicians, aged 40 to 84 at baseline in 1982.

Using a 2x2 factorial design:

- 325 mg of aspirin (Bufferin, supplied by Bristol-Myers Products on alternate days)
- 50 mg of beta-carotene (Lurotin, supplied by BASF AG on alternate days)
1\textsuperscript{st} Collapse of all-trans $\beta$-Carotene 1995
2\textsuperscript{nd} Failure of all-trans $\beta$-Carotene 2012

### CHEMOPREVENTION OF LUNG CANCER

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relative risk comparisons</th>
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<tbody>
<tr>
<td></td>
<td>Lung cancer</td>
</tr>
<tr>
<td></td>
<td>Relative Risk</td>
</tr>
<tr>
<td>ATBC</td>
<td>1.18</td>
</tr>
<tr>
<td>CARET</td>
<td>1.28</td>
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<tr>
<td>PHS</td>
<td>0.93</td>
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<tr>
<td>Linxian Study</td>
<td>—</td>
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Synthetic all-trans β-Carotene induced Cancer, Atherosclerosis & Mortality in Human from Anti-oxidant to Pro-oxidant with high medical uptake risks
Could Synthetic β-Carotene be the Real Problem?

September 1996
The Nutrition Reporter™ newsletter by Jack Challem
NIH, FDA USA recommendation 1996 – to day
“~Five Portions a Day”
of colored, fruits, vegetables
(& Algae?)

Feed
Food
Health Food
Nutraceuticals
Medicine
Fruit, Vegetable, Legume Intake and CVD Events

Aug 29, 2017

Authors: Miller V, Mente A, Dehghan M, et al., on behalf of the Prospective Urban Rural Epidemiology (PURE) Study Investigators.

Citation: Fruit, Vegetable, and Legume Intake, and Cardiovascular Disease and Deaths in 18 countries (PURE): A Prospective Cohort Study. *Lancet* 2017; Aug 29; [Epub ahead of print].

Summary By: Elizabeth A. Jackson, MD, FACC
Conclusions:

The investigators concluded that higher fruit, vegetable, and legume consumption was associated with a lower risk of cardiovascular disease and total mortality. Benefits appear to be maximum for both cardiovascular disease mortality and total mortality at 3-4 servings per day, which is equivalent to 375-500 g/day.
Dunaliella & β-Carotene
San Francisco Bay
Salt Ponds
*Dunaliella*
Dunaliella
The Great salt Lake, USA
Orange Bloom
Dunaliella
Namibia, Natural Pigmentation in Salt Lagoon, Artemia & Flamingo
Dunaliella

“Green” Micro-Algae
Dunaliella in Green Stage
Dunaliella R & D
the Weizmann Institute of Science
Dunaliella from green to orange
Biosynthesis of Carotenoids in Plants

Polar pathway

Lipophilic pathway

Acetyl-CoA + Acetoacetyl-CoA

HMG-CoA

Mevalonate

Mevalonate pyrophosphate

Isopentenyl pyrophosphate

Geranyl pyrophosphate

Farnesyl pyrophosphate

Geranylgeranyl pyrophosphate

2x

Geranylgeranyl pyrophosphate

Phytoene

Phytofluene

ζ-Carotene

Neurosporene

Lycopene

γ-Carotene

β-Carotene

STEROLS
Carotenoid Isomers in *Dunaliella* HPLC Analysis

9-cis β-carotene
The orange pigments in *Dunaliella* as in fruits & vegetables are composed of various carotenoids and stereoisomers of carotenoids.
Natural Stereoisomers of \(\beta\)-Carotene
same for all natural carotenoids

Crystals

all-trans-beta-carotene

13-cis-beta-carotene

9-cis-beta-carotene

Oil
**Dunaliella**

the richest dietary source of natural β-carotene (mg/100g)

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>all-trans</th>
<th>9-cis</th>
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<tbody>
<tr>
<td>Carrot</td>
<td>10.3</td>
<td>0.57</td>
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<tr>
<td>Lettuce</td>
<td>10.4</td>
<td>4.1</td>
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<tr>
<td>Parsley</td>
<td>12.9</td>
<td>11.1</td>
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<tr>
<td>Sweet Potato</td>
<td>11.5</td>
<td>6.5</td>
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<tr>
<td><em>Dunaliella</em></td>
<td><strong>3850.0</strong></td>
<td><strong>3780.0</strong></td>
</tr>
</tbody>
</table>

*Ben-Amotz & Fishler Food Chemistry 1998;62:515*
Dunaliella Biotechnology & Processing
Dunaliella Open Raceways & Biotechnology
NBT Ltd., Eilat, Israel, since 1987
NBT, 100,000 m²
High β-Carotene
NBT Ltd, Israel
Monzon Biotech, Spain
Algae Harvesting by Centrifuges
Dunaliella Paste
Dunaliella
Spray dried β-Carotene-rich powder
β-Carotene *Dunaliella* Powder Vacuum Packing
Japan

Israel

Towards a Sustainable Economy
Dunaliella Capsules by Nikken

300 mg dry powder/cap
20 mg β-carotene
9-cis/all-trans 1:1
Dunaliella β-Carotene Capsules
Door to door marketing by 500,000 sale agents in the Far East
Is *Dunaliella* Stereoisomeric $\beta$-Carotene Pro-Vitamin A?
Chicks Study
Vitamin A?

No Vitamin A  Vitamin A  Synthetic all-trans β-carotene  Dunaliella
Chicken Study

No Vitamin A

*Dunaliella*

\[ \beta\text{-carotene} = \text{pro-vitamin A} \]
Dunaliella Chicken Study

Control, all-trans β-carotene \textit{Dunaliella} powder

Chicken coloration by the accessories xanthophylls
Dunaliella Hard Capsules
Natural Stereoisomeric β-Carotene

Specifications:
- β-Carotene 8%
- 9-cis/all-trans, 50%
- Total bacteria less 3,000/g
- Molds, 0
- Yeasts, 0
- Heavy metals less 0.1ppm
- Pheophorbide less 100 mg%
- Toxicology & More
NBT,
Nikken Sohonsha Co
*Dunaliella bardawil*
USA FDA
“GRAS”
Sheba Medical Center
Dunaliella Research Center
β-Carotene, the Packaging Structural Model

*All-trans* (crystals, not absorbed in tissue)

*All-trans*/*9-cis* (absorbed)

*9-cis* (absorbed)
Conversion of 9-cis-β-Carotene to 9-cis & all-trans Retinoic Acids
Natural Carotenoids, Metabolic Molecular Function

9-cis β-Carotene \(\rightarrow\) 9-cis Retinoic acid, “RXR”

Dunaliella

AGGTCA TAGGTCA Target Gene

PPRE

PPAR

Metabolic Function

Medical Treatment

Nucleus
Natural β-Carotene
Molecular Cellular Medical Activity of the Isomers
9-cis (RXR) & all-trans (RAR)

RXR treated diseases:
Atherosclerosis
Diabetes
Psoriasis
Eye diseases (Retinitis pigmentosa)
Alzheimer
RXR Dunaliella Medical Papers
Dr. Aviv Shaish et al., 2000 – 2017, ~50 papers
Sheba Medical Center, Israel


9-cis beta-carotene-rich powder of the alga Dunaliella bardawil increases plasma HDL-cholesterol in fibrate-treated patients.


Treatment with 9-cis β-carotene-rich powder in patients with retinitis pigmentosa: a randomized crossover trial.

Rotenstreich Y¹, Belkin M, Sadetzki S, Chetrit A, Ferman-Attar G, Sher I, Harari A, Shaish A, Harats D.


Prevention of atherosclerosis progression by 9-cis-β-carotene rich alga Dunaliella in apoE-deficient mice.

The Highest Source of RXR is 9-cis \( \beta \)-Carotene *Dunaliella*
Biosynthesis of 9-cis β-carotene in *Dunaliella*

**Low Temp**
**High Light**
**Light Spectrum**
**Environment**
**Salt**
**???**

**Process Protection**
**Shelf Life**
New Added Commercial & Nutritional High Value Products by Dunaliella
This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613870

www.d-factoryalgae.eu
New Contribution by the Partners of the D-factory

New Strains of *Dunaliella* (MBA, UK)
Physiology & Biochemistry (UoG, UK)
Raceway technology (NBT) and Flue Gas (Monzon Biotech, Spain)
Photobioreactors (A4F, Portugal)
Centrifuge Harvesting (Evodos, the Netherlands)
Membrane Harvesting & Solvent Removal (IBET, Portugal)
scCO$_2$ extraction (NATECO, Germany)
Purification of Carotenoids by HPCCC (DE, UK)
New Products & Formulation (RISE, Sweden)
Social Assessment (RISE, Sweden)
Integrated & Environment Assessment (IFEU, Germany)
Modelling (NTUA, Greece)
Economic & Business Assessment (HI, UK)
Dissemination and Newsletter (IN, Italy)
Finance (Wendy Curran, UoG, UK)
Natural β-Carotene Future Marketing
Global β-carotene market value, by application, 2014 - 2024 (USD Million)

http://www.grandviewresearch.com/
Dunaliella  Spirulina  Haematococcus
Algal Food Colors
Global β-carotene market revenue, by source, 2014 - 2024 (USD Million)
NBT on Raceways Expansion 2020
From 10 hectares to 50 hectares
Thank You