

Risk Assessment

β-Carotene

General information

Chemistry

β-Carotene (C₄₀H₅₆) is a member of the carotenoid family of isoprenoid compounds, which are characterised by their polyunsaturated nature and antioxidant properties. The compound can exist in different geometrical forms (as *cis*- or *trans*- isomers); the majority of naturally-occurring β-carotene, as well as virtually all of the compound prepared by chemical synthesis, is the all-*trans* isomer.

Natural occurrence

β-Carotene is synthesised in plants and microorganisms, but not in higher organisms.

Occurrence in food, food supplements and medicines

The main food sources of β-carotene are yellow and green (leafy) vegetables and yellow fruits. Commercially-available β-carotene is either synthetic or derived from palm oil, algae or fungi, and is widely used as a yellow colouring agent (E160a) in foods and drinks. β-carotene is widely used in vitamin and mineral supplements at levels ranging from 0.4 mg to 20 mg per day, and is given medicinally in doses of up to 6 mg/day for dietary deficiency of vitamin A and up to 300 mg/day in the reduction of photosensitivity in individuals with erythropoietic protoporphyria (EPP).

Recommended amounts

β-Carotene *per se* is not an essential nutrient and there are currently no dietary reference values specifically for β-carotene in the UK or USA. However, as β-carotene is a precursor for vitamin A, dietary intakes are traditionally expressed as part of the RNI for vitamin A as retinol equivalents (RE). For adults the RNI for vitamin A is 600 and 700 μg RE/day (0.6 and 0.7 mg) for females and males respectively (COMA, 1991). 6 μg β-carotene = 1 μg RE.

Analysis of tissue levels and β-carotene status

β-Carotene status is assessed by measurement of plasma concentration.

Brief overview of non-nutritional beneficial effects

Observational studies in humans have shown that high intake of β-carotene-containing foods in the diet, as well as higher serum β-carotene levels, are associated with reduced risk of chronic diseases, such as coronary heart disease and cancer. It has been postulated that this association may be due to the antioxidant properties of the molecule. However, it is unknown whether β-carotene intake is acting as a marker for other components of fruits and vegetables or another lifestyle factor, rather than having an effect in its own right.

Function

β -Carotene is a precursor of vitamin A, and (as recently suggested) of other retinoid-like compounds. Its importance in any individual depends upon the level of pre-formed vitamin A in the diet.

Deficiency

β -Carotene is not classed as an essential vitamin, but it is a provitamin of vitamin A. Therefore deficiency as a clinical condition *per se*, is not clearly established.

Interactions

Interactions between β -carotene and other carotenoids (such as lycopene, lutein and canthaxanthin) occur during absorption and/or metabolism.

Absorption and bioavailability

Dietary fat and bile salts facilitate absorption in the upper small intestine, which occurs via incorporation into multilamellar lipid micelles. It has been estimated that, in humans, 10 to 90% of the total β -carotene consumed in the diet is absorbed, and that absorption decreases as intake increases. Availability from food products is lower than that of a water-dispersed formulation, due to the need for disruption (by pepsin and proteolytic enzymes and by cooking), of the matrix of fibre, polysaccharide and protein. Bioavailability is reduced in very low fat diets.

Distribution and metabolism

A proportion of absorbed β -carotene is converted to retinol within intestinal mucosal cells. Unaltered β -carotene is transported via the lymph to the plasma where it is associated with lipoproteins. Tissue uptake and distribution are not well characterised. In the case of regular high intake, long-term accumulation occurs preferentially in adipose tissues.

Serum levels of β -carotene have been reported to be low in smokers, in individuals with a high alcohol intake, and in those with HIV infection. Low β -carotene status may be associated with conditions of impaired lipid absorption such as jaundice, liver cirrhosis and cystic fibrosis.

β -Carotene is mainly converted to retinol (vitamin A) in the cytosol of intestinal mucosal cells. Experiments in rats have shown that the process is regulated by the levels of β -carotene and of pre-formed vitamin A. *In vitro* studies have shown that other β -carotene derivatives may also occur, but their biological activity, and whether they are synthesised *in vivo*, is unknown.

Carotenoid absorption and metabolism vary considerably between animal species. No single species provides a good model for studying all aspects of the biokinetics and metabolism of β -carotene in humans. The rat is particularly unsuitable, due to the high efficiency of conversion to vitamin A, such that significant levels of unaltered β -carotene are absorbed only when very high doses are given, for prolonged periods of time. The pre-ruminant calf, the ferret and the Mongolian gerbil are suggested to be more useful models, although it is apparent that there are many differences between carotenoid absorption, distribution and metabolism in these animals and humans.

Excretion

Absorbed β -carotene is secreted into the bile and excreted in the faeces. It is also excreted in the sweat.

Toxicity

Human data

Until recently, β -carotene was considered to show no toxicity in humans. Hypercarotenaemia (high plasma β -carotene) has not been associated with adverse effects other than reversible yellowing of the skin (hypercarotenodermia). Long-term, oral β -carotene therapy, in doses up to 300 mg/day has shown no toxic effects in individuals with erythropoietic protoporphyria (EPP). Vitamin A toxicity does not occur, as the metabolic conversion is regulated by vitamin A status. There have been no reports of reproductive toxicity or teratogenicity associated with high β -carotene intake, either before or during pregnancy. Two recent, large-scale supplementation trials testing the hypothesis that β -carotene supplementation in smokers would reduce the incidence of cancer, have shown an association of high-dose β -carotene supplementation (20 – 30 mg/day) with increased incidence of lung cancer in smokers and asbestos-exposed individuals. No statistically significant differences in other cancer types were observed in these studies. These are described in more detail below.

Supplementation studies

Two large-scale studies (Alpha-Tocopherol Beta-Carotene Prevention Study (ATBC) and β -Carotene and Retinol Efficacy Trial (CARET)) have shown an association of β -carotene supplementation (20 mg/day, alone or in combination with α -tocopherol; 30 mg/day in combination with retinyl palmitate respectively) with increased incidence of lung cancer in smokers and individuals with previous high-level exposure to asbestos. However, another large-scale study involving β -carotene supplementation in a well-nourished population, the US Physicians' Health Study, showed no beneficial or adverse effects of β -carotene supplementation (50 mg every other day), in a trial population which comprised 11 % current smokers. Additionally, the Heart Protection Study, a large scale study of supplementation with 20 mg β -carotene, 60 mg vitamin E and 250 mg vitamin C daily for up to 5 years, showed no difference in cancer incidence compared to placebo controls. The designs of these studies are given below:

Table. Study design, treatment (type, dose and duration) and predefined end-points in four major intervention trials involving β -carotene (adapted from International Agency for Research on Cancer, 1998)

	ATBC ¹	CARET ²	PHS ³	Heart Protection Study ⁴
Study design	2 x 2 factorial: 3 treatment and 1 placebo groups	Randomised, double- blind, placebo- controlled: 1 treatment and 1 placebo groups	2 x 2 factorial: 3 treatment and 1 placebo groups	Randomised, double- blind, placebo- controlled trial: 1 treatment group and 1 placebo group
Number of subjects recruited	29,133	18,314	22,071	20,536
Treatment and dose	Daily: a-tocopherol 50mg or β -carotene 20mg or a-tocopherol + β -carotene	Daily: Retinol 25 000 IU + β -carotene 30mg	On alternate days: Aspirin 325mg or β -carotene 50mg or aspirin + β -carotene	Daily: β -carotene 20 mg, vitamin E 60 mg and vitamin C 250 mg
Duration	6 years (median)	4 years (mean)	11-12 years (for β -carotene)	Up to 5 years
Predefined end-points	Lung cancer and other major cancers, incidence	Lung and other cancers, incidence	Cardiovascular disease and lung cancer, incidence	Major coronary events, subsidiary assessments of cancer incidence and other morbidity
Results	Increased lung cancer in β -carotene treated individuals	Increased lung cancer in β -carotene treated individuals	No treatment related effect on cancer	No significant effects on incidence of vascular disease, cancer or other major outcome

1 ATBC, Alpha-Tocopherol Beta-Carotene Prevention Study (The Alpha Tocopherol Beta Carotene Prevention study group, 1994)

2 CARET, β -carotene and Retinol Efficacy Trial (Omenn *et al.*, 1996b)

3 US Physicians' Health Study (Hennekens *et al.*, 1996)

4 MRC/BHF Heart Protection Study of antioxidant vitamin supplementation (Heart Protection Study Collaborative Group, 2002)

Smaller-scale studies, involving varying levels of β -carotene supplementation, up to 300 mg/day, have shown no evidence of toxicity.

Animal data

No adverse effects of high-dose β -carotene supplementation have been observed in standard toxicological studies in experimental animals. These include tests for acute toxicity (doses up to 5000 mg/kg bw/day in rats), chronic toxicity/carcinogenicity (doses up to 1000 mg/kg bw/day for life in rats and mice; up to 250 mg/kg bw/day for 2 years in beagle dogs) and teratogenicity and reproductive toxicity (doses up to 1000 mg/kg bw/day for 3 generations, or during days 7 to 16 of gestation, in rats; up to 400 mg/kg bw/day during days 7 to 19 of gestation in rabbits).

Carcinogenicity and genotoxicity

β -Carotene shows no genotoxicity *in vitro* or at high doses *in vivo* and was not carcinogenic in experimental rodent studies. However, β -carotene supplementation (2.4 mg/kg bw/day, with or without exposure of the animals to cigarette smoke) was associated with the development of squamous cell metaplasia in the lungs of ferrets (Wang *et al.*, 1999).

Vulnerable groups

Groups vulnerable to β -carotene toxicity include current smokers, individuals with previous high-level exposure to asbestos, those with high alcohol intakes, and/or a history of myocardial infarction.

Genetic variations

No genetic variations that increase sensitivity to β -carotene toxicity have been identified.

Mechanism of toxicity

A number of hypotheses have been suggested to account for the association between β -carotene supplementation and lung tumourigenesis in smokers, including an imbalance of other carotenoids or antioxidant species, a pro-oxidant activity of β -carotene at high oxygen tensions (in the lungs), induction of P450 enzymes and the production of damaging β -carotene oxidation products by components of cigarette smoke. However, the mechanism for the observed association remains to be established.

Dose-response characterisation

20 mg/day β -carotene was associated with increased incidence of lung cancer in human smokers and individuals with previous high-level exposure to asbestos. Similar studies with lower doses have not been carried out.

Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD)

Toxicity in humans

The Alpha-Tocopherol, Beta-Carotene (ATBC) Trial (The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study Group, 1994; Albanes et al., 1996, Rapola et al., 1997)

This was a randomised, double-blind, placebo-controlled, 2x2 factorial trial to assess the potential efficacy of daily supplementation with α -tocopherol and/or β -carotene in the prevention of lung cancer. Finnish, male smokers (n = 29,133) were given a daily supplement of 50 mg α -tocopherol, 20 mg β -carotene, 50 mg α -tocopherol + 20 mg β -carotene or placebo for a period of 5-8 years. Statistical

analysis for a median follow-up period of 6.1 years showed no statistically significant change in the primary assessed endpoint, lung cancer incidence, in the α -tocopherol-only treatment group, but a significant 18% increase was noted in men who received β -carotene supplementation compared with those who did not. There was no evidence of an interaction between α -tocopherol and β -carotene in their effects on lung cancer. No significant changes were seen in the incidences of cancers other than lung cancer, but there was a significant increase in the risk of fatal coronary heart disease in individuals with a previous myocardial infarction in the β -carotene-treated groups.

The Beta-Carotene and Retinol Efficacy Trial (CARET) (Omenn et al., 1996a,b).

This was a multicentre, randomised, double-blind, placebo-controlled primary prevention trial of β -carotene and retinol supplementation in individuals considered to be at high risk of developing lung cancer (14,254 male and female heavy smokers and 4060 males with previous high-level occupational asbestos exposure). The aim of the investigation was to assess the effects of daily supplementation with a combination of 30 mg β -carotene + 25 000 IU retinol (as retinyl palmitate), compared with a placebo, upon the primary measured endpoint – the incidence of lung cancer. The trial was terminated approximately 2 years prematurely (after a mean intervention time of 4 years) when a trend towards increased incidence of cancer in participants receiving supplementation with β -carotene became evident. At this time, statistical analysis showed a significant 28 % increase in the relative risk of lung cancer in the supplementation group compared with the placebo group. No statistically significant differences in other cancer types were observed.

The US Physicians' Health Study (US-PHS) (Hennekens et al., 1996).

This was a randomised, double-blind, placebo-controlled, 2x2 factorial trial involving 22,071 male physicians (of whom 11% were current smokers) to assess the effects of aspirin and/or β -carotene supplementation on the incidence of cardiovascular disease and cancer. Supplementation, on alternate days, of either 325 mg aspirin, 50 mg β -carotene, 325 mg aspirin + 50 mg β -carotene, or placebo, was given for 6 years, at which time aspirin supplementation was discontinued whilst the randomised β -carotene component of the trial continued for another 6 years. After an average 12 years of follow-up, statistical analysis showed no significant effect of β -carotene supplementation on overall cancer incidence, the incidences of specific cancer types (including lung cancer), myocardial infarction, stroke, death due to cardiovascular pathology, all important cardiovascular events, or death from all causes. It has been suggested that, as current smokers comprised only 11% of the total study population the PHS trial had limited capacity to detect adverse effects in this subgroup (in contrast with ATBC where 100% of the study population were smokers, and CARET, which included only current or recently-quit heavy smokers and individuals with previous high-level asbestos exposure).

Heart Protection Study Collaborative Group, 2002

20,536 adults with coronary artery disease, other occlusive arterial disease or diabetes were allocated to receive either 20 mg β -carotene/day, together with 600 mg/day vitamin E and 250 mg/day vitamin C, or placebo for up to 5 years in this randomised, placebo controlled trial. Small but statistically significant increases in total plasma cholesterol, LDL cholesterol and triglycerides were observed in patients on the active treatment compared to controls. However, no significant differences in the incidences of all-caused mortality, or in deaths due to vascular or non-vascular events, were observed. Nor were there any significant differences in the incidence of non-fatal myocardial infarctions or coronary death, non-fatal or fatal strokes, coronary or non-coronary revascularisation, or in the incidence of cancer or of hospitalisation for non-cancer causes.

Toxicity in animals

The vast majority of standard toxicological studies in experimental animals have shown no adverse effects of high dose β -carotene supplementation. One study (Wang *et al.*, 1999) has described an association of β -carotene supplementation with the development of squamous metaplasia in the lungs of ferrets:-

Wang et al., 1999

This study used a ferret model to assess the effects of cigarette smoke and β -carotene supplementation, alone and in combination, on lung histopathology/biochemistry. Four groups of 6 males were treated with dietary β -carotene supplementation (2.4 mg/kg bw/day), cigarette smoke exposure, both, or neither, for a period of 6 months. Histopathological analysis revealed that all β -carotene treated animals showed an increase in cell proliferation and squamous metaplasia in lung tissue, and this was further enhanced in the animals that were also exposed to cigarette smoke. Animals exposed to cigarette smoke alone, did not show these changes. The histopathological endpoint, squamous metaplasia, may not, however, be directly related to carcinogenesis (Lotan, 1999). Furthermore, these findings are not consistent with the results of human studies, in which β -carotene supplementation showed no effect on sputum atypia or metaplasia in smokers or asbestos workers (McLarty *et al.*, 1995; van Poppel *et al.*, 1997).

Exposure assessment

Total exposure/intake

Food ²²	Mean: 2.3 mg/day in adults 97.5th percentile: 7.0 mg/day (NDNS, 1986/7)
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Supplements	up to 20 mg/day (Annex 4)
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Estimated maximum daily intake: $7.0 + 20 = 27$ mg

No potential high intake groups have been identified.

Risk assessment

β -carotene is of low toxicity in both animals and man, and prior to the publication of a number of intervention studies was thought to be without adverse effect, other than a yellowing of the skin, which occurred after sustained high intake. However, supplementation of smokers and subjects previously exposed to asbestos has been associated with an increased risk of lung cancer. The mechanism for this effect is unknown but it seems likely that β -carotene has a tumour promoting effect of some type.

²² Note: intake data quoted for food are for total carotene content

ESTABLISHMENT OF SAFE UPPER LEVEL

Key study:	The Alpha Tocopherol and Beta Carotene Cancer Prevention Study Group (1994).
Effects observed at:	20 mg/person/day supplemental β -carotene.
Uncertainty Factor:	3 (for LOAEL to NOAEL extrapolation).
Safe Upper Level for daily consumption over a lifetime:	$20/3 = 7$ mg/day supplemental β -carotene (equivalent to 0.11 mg/kg bw/day in a 60 kg adult)

Epidemiological studies have shown an association between supplementation with β -carotene and an increase in lung cancers in smokers and in individuals who have been heavily exposed to asbestos. The Safe Upper Level applies only to the general population, *i.e.* non-smokers and those not exposed to asbestos.

There is no evidence that β -carotene supplementation has any effect on non-smokers. However, until the mechanism for the promotion of lung tumours is established it remains uncertain whether other co-exposures could have the same effect as observed in smokers. As a matter of prudence, therefore, the EVM has set a Safe Upper Level for supplementation, based on the ATBC study. This study was chosen for use in deriving a Safe Upper Level, as of the four large-scale studies investigating the effects of β -carotene supplementation on smokers and/or those exposed to high levels of asbestos, this study has shown effects at the lowest level of supplemental intake. The LOAEL from this study was 20 mg/day. Applying an uncertainty factor of 3, to extrapolate from a LOAEL to a NOAEL, results in a Safe Upper Level for supplementation of 7 mg/day. This is equivalent to 0.11 mg/kg bw day for a 60 kg adult. This Safe Upper Level applies to supplements only, as there is no evidence to suggest that current levels of β -carotene intake from food results in adverse effects.

The dose at which supplemental β -carotene promotes carcinogenesis in smokers and workers exposed to asbestos is unknown. However, there is no evidence that dietary β -carotene is associated. The EVM recommends that, as a matter of prudence, smokers or those exposed to asbestos should not take β -carotene supplements.

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