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Carotenoids and Cancer: An Update with Emphasis on Human Intervention Studies

Geert van Poppel

This article gives an overview of the current state of knowledge on the cancer preventive potential of carotenoids. Numerous retrospective and prospective epidemiological studies have shown that a high intake of carotenoid-rich fruits and vegetables is associated with a decreased risk of cancer at a number of common sites. For several other cancer sites, however, the epidemiological evidence is not very consistent. A number of mechanisms for the cancer preventive properties of carotenoids have been proposed. Conversion to retinol, possibly in posthepatic tissues, would allow an effect on cellular differentiation and proliferation, and on cell-to-cell communication. Antioxidant functions could prevent free radical-induced damage to cellular DNA and other macromolecules. Immunomodulatory effects could enhance immune surveillance in tumorigenesis. In addition, non-retinol-mediated effects of carotenoids on metabolism of carcinogens and cell-to-cell communication have been shown. Observational epidemiology cannot resolve whether associations are due to a specific carotenoid, or to an associated factor in fruits and vegetables, whereas interpretation of animal studies is hampered by uncertainties in extrapolation between species, more so because the metabolism of carotenoids in most animals differs notably from that in humans. Human intervention studies on biomarkers related to cancer risk and on cancer incidence are, therefore, necessary. Human intervention studies performed so far suggest that β-carotene can affect carcinogenesis, though not at all stages and not at all cancer sites. Implications for future human intervention research are discussed.
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INTRODUCTION

In 1981, Peto *et al.* [1] hypothesised that dietary carotenoids may reduce human cancer rates. Since then, a large number of epidemiological studies have addressed this topic, and a flurry of experimental work has been aimed at unravelling the possible mechanisms of chemoprevention by carotenoids. This article will give an overview of the current state of knowledge regarding the cancer preventive potential of carotenoids. Firstly, an update on epidemiological studies regarding carotenoids and cancer will be given. Subsequently, the current concepts on mechanisms of carcinogenesis will be addressed and possible mechanisms of action of carotenoids will be discussed. Finally, results of human intervention studies that have so far been performed will be addressed with implications for future research.

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EPIDEMIOLOGICAL STUDIES ON CAROTENOIDS AND CANCER

A large number of case–control studies have evaluated the association between intake of fruits and vegetables containing carotenoids and cancer. Likewise, prospective cohort studies have evaluated the relation between prediagnostic consumption or blood levels of carotenoids and subsequent risk of cancer. Tables 1 and 2 summarise the results for the retrospective and prospective studies. Several points should be considered when

Table 1. Retrospective studies on estimates of dietary intake of carotenoids and cancer, grouped by site

Site	First author*	Year	No. of cases	Exposure measure	Association	Relative risk High vs. lowest	Population
Lung	MacLennan	1977	233	Green vegetable index	↓	0.45	Men and women, Singapore Chinese
	Kolonel	1983	267	Carotenoids	↓	0.40	Men and women, multiethnic, Hawaii
	Hinds	1984	364	Carotenoids	↓	0.45	Men and women, multiethnic, Hawaii
	Wu	1985	216	β-Carotene	↓	0.40	White women, Los Angeles County
	Samet	1985	447	Carotenoids	n.s.	0.76	White men and women, New Mexico
	Ziegler	1986	763	Carotenoids	↓	0.59	White men, New Jersey
	Pisani	1986	417	Carrots/green vegetables	↓	0.50	Men and women, northern Italy
	Bond	1987	308	Carotenoid	n.s.	0.42	Chemical employees, Texas
	Byers	1987	450	Carotenoids	↓	0.63	Men and women, New York
	Pastorino	1987	47	Carotenoids	n.s.	0.34	Women, northern Italy
	Koo	1988	88	Carotenoid-rich vegetables	n.s.	0.60	Chinese women, Hong Kong
	Fontham	1988	1253	Carotenoids	n.s.	0.88	Men and women, southern Louisiana
	Le Marchand	1989	332	β-Carotene	↓	0.53	Men and women, multiethnic Hawaii
	Jain	1990	839	β-Carotene	n.s.	0.89	Men and women, Toronto area
	Dartiques	1990	106	Carotenoids	↓	0.25	Men and women, south-western France
	Harris	1991	96	Carotenoids	↓	0.45	Men, U.K.
	Wu-Williams	1991	965	Carotene-rich vegetables	n.s.	0.90	Women, northeast China
Oesophagus	Ziegler	1981	120	Carotene	n.s.	0.77	Black men, Washington DC
	Tuyns	1987	743	Carotene	↓	0.47	Men and women, Calvados, France
	Decarli	1987	105	β-Carotene	↓	0.23	Men and women, northern Italy
	Brown	1988	209	Carotene	n.s.	0.80	Men, South Carolina
	Graham	1990	178	Carotene	n.s.	0.66	Men and women, New York
Oral cavity	Winn	1984	227	Fruits and vegetables	↓	0.5	Black and white women, southern U.S.A.
Pharynx	McLaughin	1988	871	Carotene	n.s.	0.9; 0.8 f	White men and women, U.S.A.
	Rossing	1989	166	Carotenoids	n.s.	1.0	Men and women, Washington State
	Ning	1990	100	Carrots	n.s.	0.4	Men and women, Tianjin, China
Stomach	Correa	1985	391	Carotenoids	n.s.	0.68; 1.08	Men and women, Louisiana, (white; black)
	Risch	1985	246	β-Carotene	↓	0.33	Men and women, Canada
	Jedrichowski	1986	110	Fruits and vegetables	↓	0.24	Men and women, Cracow, Poland
	La Vecchia	1987	206	β-Carotene	↓	0.39	Men and women, northern Italy
	You	1988	564	Carotene	↓	0.50	Chinese men and women, rural Shandong
Pancreas	Gold	1985	201	Raw fruits and vegetables	↓	0.55	Men and women, Baltimore area
	Norell	1986	99	Vegetables	n.s.	0.5	Men and women, Sweden
	Falk	1988	363	Carotenoids	n.s.	0.82 m; 1.65 f	Men and women, Louisiana
	Olsen	1989	212	Vegetables	n.s.	0.95	White men, Minneapolis area

Continued over

interpreting these epidemiological studies. Firstly, neither dietary intake nor plasma level studies can resolve whether associations are due to specific carotenoids or to associated components of fruits and vegetables. Secondly, misclassification by rather crude assessment of dietary intake may have attenuated associations. Thirdly, the retrospective case–control studies may suffer from recall bias in patients. Finally, in the studies using plasma levels, a crude adjustment for number of cigarettes smoked may not adequately account for the known metabolic effects of smoking on β-carotene [2].

Table 1. Continued

Site	First author*	Year	No. of cases	Exposure measure	Association	Relative risk High vs. lowest	Population
Colon, rectum	Macquart-Moulin	1986	399	Vegetables	↓	0.42 m; 0.68 f	Men and women, Marseille region
	Potter	1986	419	β-Carotene	n.s.	0.8 m; 2.2 f	Men and women, South Australia
	Kune	1987	715	β-Carotene	↓	0.45	Men and women, Melbourne
	Graham	1988	428	Carotene	n.s.	—	Men and women, New York
	La Vecchia	1988	575	Green vegetables	↓	0.5	Men and women, northern Italy
	West	1989	231	β-Carotene	↓	0.4 m; 0.6 f	Men and women, Utah
	Freudenheim	1990	423	Carotenoids	↓	0.59 m; 0.70 f	Men and women, New York
	Peters	1989	147	Raw fruits and vegetables	↓	0.59	Men and women, Los Angeles County
	Young	1988	353	Yellow vegetables	n.s.	0.78	White men and women, Wisconsin
	Slattery	1988	231	Vegetables	n.s.	0.6	White men and women, Utah
	Bidoli	1992	148	Vegetables	n.s.	0.6	Men and women, northern Italy
Bladder	Dunham	1968	493	Leafy and yellow vegetables	n.s.	—	Black and white men and women, Louisiana
	Mettlin	1979	569	Carrots	↓	0.62	Men and women, New York State
	La Vecchia	1989	163	Carotenoids	↓	0.41	Men and women, northern Italy
	Risch	1988	826	β-Carotene	n.s.	0.95	Men and women, Canada
	Claude	1986	431	Fruits and vegetables	↓ m	0.59 m; 0.90 f	Men and women, northern Germany
Cervix	Marshall	1983	513	β-Carotene	↓	0.50	White women, New York
	Brock	1988	117	β-Carotene	↓	0.5	Women, Sydney, Australia
	La Vecchia	1988	392	β-Carotene	n.s.	0.18; 1.09	Women, northern Italy (invasive; intraepithelial)
	Verreault	1989	189	Carotenoids	n.s.	0.6	Women, Washington State
	Ziegler	1990	271	Carotenoids	n.s.	0.98	White women, U.S.A.
	VanEenwijk	1991	102	β-Carotene	n.s.	0.56	Women, Chicago, U.S.A.
Breast	La Vecchia	1987	1108	Green vegetables	↓	0.42	Women, northern Italy
	Iscovich	1989	150	β-Carotene	n.s.	0.92	Women, Argentina
	Katsouyanni	1988	120	Carotene	↓	0.56	Women, Greece
	Rohan	1988	451	β-Carotene	↓	0.76	Women, South Australia
	Marubini	1988	214	β-Carotene	n.s.	1.20	Women, northern Italy
	Toniolo	1989	250	β-Carotene	n.s.	1.00	Women, northern Italy
	Van't Veer	1990	133	β-Carotene	n.s.	0.73	Women, Netherlands
	Potischman	1990	83	Carotenoids	n.s.	0.81	Women, New York State
	Richardson	1991	409	β-Carotene	n.s.	1.0	Women, Montpellier, France
Ovary	Slattery	1989	85	β-Carotene	↓	0.50	White women, America
	Byers	1983	274	Carotenoids	n.s.	0.77	Women, New York
	La Vecchia	1987	455	Carotene	n.s.	0.94	Women, northern Italy
	Shu	1989	172	Carotene	n.s.	1.0	Women, Shanghai, China
Prostate	Ohno	1988	100	β-Carotene	↓	0.34	Men, Japan
	Ross	1987	142	β-Carotene	n.s.	0.6; 1.0	Men, California (black; whites)
	Mishina	1985	100	Green and yellow vegetables	n.s.	0.5	Men, Japan
	Talamini	1986	166	Green vegetables	n.s.	1.20	Men, Pordenone, Italy
	Mettlin	1989	371	β-Carotene	↓	0.60	Men, New York
	Oishi	1988	100	β-Carotene	↓	0.47	Men, Japan
	Le Marchand	1991	452	β-Carotene	n.s.	—	Men, multiethnic, Hawaii

* A complete list of references is available from the author.
n.s. = Not statistically significant. f = in females only. m = in males only.

The results of the case–control studies show that high intake of fruits and vegetables that are rich in carotenoids have been associated with decreased risk of cancer at a number of common sites. This association appears to be most consistent for lung and stomach cancer, and least consistent for breast, prostate, oesophageal and oral cancer. The prospective studies are remarkably consistent for an inverse relation of carotenoids with lung cancer. For stomach cancer, the prospective evidence is similar, though the magnitude and the number of studies is modest. For breast and prostate cancer, the prospective studies are in line with the retrospective studies and do not indicate a clearly consistent association of plasma or dietary carotenes with

Table 2. Prospective studies on dietary intake or blood levels of carotenoids and cancer, grouped by site

Site	First author*	Year	No. of cases	Exposure measure	Association	Relative risk High vs. lowest	Population
Lung	Shekelle	1981	33	Carotenoid intake	↓	0.14	Men, Western Electric Study
	Long-de	1985	2952	Green salad and fruit	↓	0.56	Men and women, China
	Hirayama	1986	1917	Green-yellow vegetables	↓	0.79 m; 1.35 f	Men and women, Japan
	Kromhout	1987	63	Carotenoid intake	n.s.	0.68	Men and women, Zutphen, The Netherlands
	Paganini-Hill	1987	56	Carotenoid intake	n.s.	0.72 m; 0.67 f	Men and women, Leisure World, Los Angeles
	Knekt	1991	108	Carotenoid intake	↓	0.40; 0.93	Men, Finland (non smokers; smokers)
	Fraser	1991	61	Fruit and green salad	↓	0.26; 0.65	White men and women, California Adventists (fruit; salad)
	Willett	1984	17	Plasma total carotene	n.s.	—	Men and women, U.S.A., hypertension follow-up
	Connett	1989	66	Plasma β-carotene	↓	0.43	Men, U.S.A., MRFIT Study
	Nomura	1985	74	Plasma β-carotene	↓	0.29	Men, Japanese ancestry, Hawaii
	Wald	1988	50	Plasma β-carotene	↓	0.41	Men, U.K., BUPA Study
	Stähelin	1991	64	Plasma total carotene	↓	0.56	Men and women, Basel Study
	Knekt	1990	108	Plasma β-carotene	n.s.	1.00	Men and women, Finland
	Comstock	1991	99	Plasma β-carotene	↓	0.45	Men and women, Washington County
	Orentreich	1991	123	Plasma β-carotene	↓	0.33	Men and women, U.S.A.
Stomach	Hirayama	1986	5247	Green and yellow vegetables	↓	0.66 m; 0.66 f	Men and women, Japan
	Nomura	1985	70	Plasma β-carotene	n.s.	—	Men, Japanese ancestry, Hawaii
	Wald	1988	13	Plasma β-carotene	n.s.	—	Men, U.K., BUPA Study
	Knekt	1990	32	Plasma β-carotene	n.s.	0.8	Men and women, Finland
	Stähelin	1991	16	Plasma total carotene	↓	0.34	Men and women, Basel Study
Pancreas	Knekt	1990	10	Plasma β-carotene	n.s.	0.60	Men and women, Finland
	Mills	1988	40	Green vegetables and salad	n.s.	—	Men and women, California Adventists
	Comstock	1991	22	Plasma β-carotene	n.s.	0.83	Men and women, Washington County
Colon, rectum	Heilbrun	1989	162	Dietary β-carotene	n.s.	0.72	Men, Japanese ancestry, Hawaii
	Paganini-Hill	1987	110	Dietary carotenoids	n.s.	0.90 m; 1.17 f	Men and women, Leisure World, Los Angeles
	Shekelle	1981	49	Dietary carotenoids	n.s.	—	Men, Western Electric Study
	Comstock	1991	106	Plasma β-carotene	n.s.	0.83; 1,25	Men and women, Washington County (colon, rectum)
	Connett	1989	14	Plasma β-carotene	n.s.	—	Men, U.S.A., MRFIT Study
	Nomura	1985	113	Plasma β-carotene	n.s.	—	Men, Japanese ancestry, Hawaii
Colon, rectum	Schober	1987	72	Plasma β-carotene	n.s.	0.83	White men and women, Washington County
	Wald	1988	30	Plasma β-carotene	n.s.	—	Men, U.K., BUPA Study
	Knekt	1990	13	Plasma β-carotene	n.s.	0.30	Men and women, Finland
	Stähelin	1991	32	Plasma total carotene	n.s.	0.76	Men and women, Basel Study
Bladder	Knekt	1990	18	Plasma β-carotene	n.s.	0.30	Men and women, Finland
	Paganini-Hill	1987	59	Dietary carotenoids	↓	0.62 m; 0.15 f	Men and women, Leisure World, Los Angeles
	Shekelle	1981	19	Dietary carotenoids	n.s.	—	Men, Western Electric Study
	Nomura	1985	27	Plasma β-carotene	n.s.	—	Men, Japanese ancestry, Hawaii
	Wald	1988	15	Plasma β-carotene	n.s.	—	Men, U.K., BUPA Study
	Comstock	1991	35	Plasma β-carotene	n.s.	0.63	Men and women, Washington County
Breast	Hirayama	1979	142	Green and yellow vegetables	n.s.	—	Women, Japan
	Paganini-Hill	1987	123	Dietary carotenoids	n.s.	0.83	Men and women, Leisure World, Los Angeles
	Comstock	1991	30	Plasma β-carotene	n.s.	0.9	Women, Washington County, postmenopausal
	Wald	1984	39	Plasma β-carotene	n.s.	0.35	Women, Guernsey, U.K.
	Willett	1984	14	Plasma total carotene	n.s.	—	Women, U.S.A., hypertension follow-up

Continued overleaf

Table 2. Continued

Site	First author*	Year	No. of cases	Exposure measure	Association	Relative risk High vs. lowest	Population
Prostate	Hirayama	1986	183	Green and yellow vegetables	n.s.	1.23	Men, Japan
	Mills	1988	180	Fruits and salads	↓	0.70; 0.68	Men, California Adventists (fruits; salads)
	Snowdown	1984	247	Fruits and vegetables	n.s.	—	Men, Seventh Day Adventists
	Paganini-Hill	1987	93	Dietary carotenoids	n.s.	0.98	Men and women, Leisure World, Los Angeles
	Shekelle	1981	29	Dietary carotenoids	n.s.	—	Men, Western Electric Study
	Comstock	1991	103	Plasma β-carotene	n.s.	0.91	Men, Washington County
	Willett	1984	11	Plasma total carotene	n.s.	—	Men, U.S.A., hypertension follow-up
	Knekt	1990	32	Plasma β-carotene	↓	0.20	Men, Finland
Skin	Shekelle	1981	36	Dietary carotenoids	n.s.	—	Men, U.S.A., Western Electric Study
	Wald	1988	56	Plasma β-carotene	n.s.	—	Men, U.K., BUPA Study
	Knekt	1991	38	Plasma β-carotene	n.s.	0.32	Men and women, Finland

* A complete list of references is available from the author.
n.s. = Not statistically significant. f = females; m = males.

reduced cancer risk. For the other cancer sites, the number of cases are often small, implying that only very strong associations would have been detected in the prospective studies.

CURRENT CONCEPTS OF CARCINOGENESIS

The traditional two-stage view of carcinogenesis, initiation followed by promotion, is derived primarily from animal models of chemical carcinogenesis. Tumour initiation involves exposure of normal cells to chemical, physical or microbial carcinogens that cause a genetic change. The altered genotype of the initiated cell is considered irreversible, but the initiated phenotype is not fully expressed except in the presence of a promotor. Promotors both enhance the expression of the initiated cell phenotype and provide a selective growth stimulus to cells expressing this phenotype, allowing clonal expansion of the initiated cells. This second stage of carcinogenesis, tumour promotion, does not involve a genetic change and is considered reversible [3].

The classical view of two-stage carcinogenesis involving a mutation (initiation) and an epigenetic change (promotion) has been conceptually important but is also considered to be simplistic in that the number of independent genetic and epigenetic changes may be six or more in certain types of cancer [4]. Carcinogenesis is thus considered as a multistage process; subsequent genetic and epigenetic changes allow susceptible cells to gain a growth advantage and undergo clonal expansions [4]. This probably involves activation of protooncogenes and/or inactivation of tumour suppressor genes (for a review, see [5]). A simplified scheme of current concepts of carcinogenesis is given in Fig. 1.

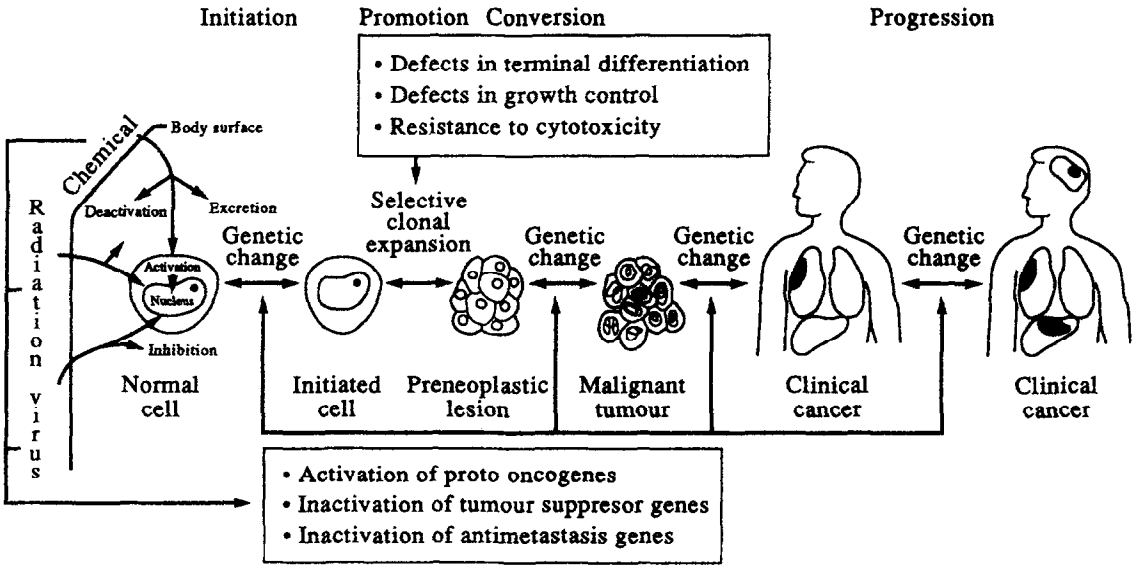

Fig. 1. A multi stage model for carcinogenesis involving multiple genetic and epigenetic events. (Reproduced with permission from *Cancer Research* [4]).

POSSIBLE CANCER PREVENTIVE ACTIVITIES OF CAROTENOIDS

Conversion to vitamin A

Less than 10% of all 600 characterised carotenoids serve as precursors for vitamin A. In humans, β-carotene, α-carotene and cryptoxanthin are converted to vitamin A, whereas lutein and lycopene are not [6]. The enzymatic conversion of carotenoids to retinal is considered to occur almost solely in the intestine and the liver [6]. Blood levels of carotenoids reflect dietary intake, whereas blood levels of retinol are under homeostatic control. Almost all epidemiological studies that observed reduced cancer risk with increasing β-carotene intake did not observe similar associations with preformed vitamin A intake [7]. The protective action of β-carotene has, therefore, been considered not to be connected with its conversion to retinol. However, De Vet [8] has argued that epidemiological studies indicating protection through β-carotene are most consistent for sites involving mainly epidermoid cancers, which is in line with the effects of vitamin A on epithelial differentiation and proliferation. De Vet [8] has, therefore, hypothesised that β-carotene may exert an influence through local conversion into retinol, i.e. *after* reaching posthepatic tissues. This possibility is supported by experiments in rats that showed conversion to retinol of intravenously administered β-carotene after removal of the liver, intestine and several other organs [9], and by recent work by Wang *et al.* [10]. Also, Edes *et al.* [11] observed that administration of the carcinogen benzo[a]pyrene induced lower tissue levels of retinol in rats. This reduction of retinol was pevented by administration of β-carotene, but not retinol. These experiments could indicate that β-carotene is capable of rapidly compensating localised vitamin A deficiencies induced by carcinogens.

The mechanisms through which retinoids may influence carcinogenesis are well documented [12, 13]. These mechanisms have been hypothesised to include an action on the cell nucleus, involving the expression of genetic information controlling cell differentiation. Specific binding proteins for retinol and retinoic acid are believed to be responsible for the transport of retinol and retinoic acid within the cell and across the nuclear membrane, suggesting a hormone-like control of cell differentiation. In addition, retinol has a variety of effects on the cell membrane, involving altered glycoprotein synthesis and changes in membrane receptors for various hormones, including those mediated by c-AMP. The action on these receptors may influence cell–cell interactions, cell adhesion and cell membrane permeability. Finally, animal studies have shown that retinol increases both the humoral and cell-mediated immune response [14]. Retinoids thus seem to influence only epigenetic changes, implying an influence primarily in the promotional stages of carcinogenesis. In accordance, an antagonistic effect of retinoids on tumour promotors has been frequently reported in animal studies [13].

Antioxidant functions

The antioxidant functions of carotene are attributed to their molecular structure. This structure would enable them to quench light-energised states of molecules, such as singlet oxygen and to neutralise free radicals that are highly reactive since they contain a non-paired electron [15]. Free radical species can result from photochemical reactions and from oxidant stress, e.g. induced by cigarette smoking, but free radicals are also a result of normal cell metabolism [16]. Free radicals are capable of initiating lipid peroxidation by reactions with polyunsaturated fatty acids, inactivating proteins and enzymes by reactions with amino acids, and damaging RNA and DNA by reactions with guanine. These reactions may occur if the cell is insufficiently protected by enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include catalase, superoxide dismutase and the selenium-dependent glutathione peroxidase. Non-enzymatic antioxidants include vitamins C and E and carotenoids [16]. β-Carotene may exert its antioxidant function through its ability to interact with a radical to yield a resonance stabilised, and thus less reactive, carbon centered radical species ([17]; Fig. 3). However, very little is as yet known about the chemistry of reactions of carotenoids with radicals. β-Carotene functions as an antioxidant in many, but not all *in vitro* systems [18]. In this respect, the evidence that β-carotene is a good radical-trapping antioxidant only at low oxygen partial pressures [17] is of interest, since low oxygen pressures are found in most tissues under physiological conditions.

With respect to carcinogenesis, antioxidants have been implied in both the initiation and promotion phases in animal studies [13]. Antioxidants may prevent genetic changes by preventing DNA damage directly induced by free radicals [19] or can hypothetically interfere with the metabolic activation of chemical carcinogens [20]. Hypotheses on the role of free radicals and antioxidants in tumour promotion [19, 21] come from observations in animal studies that (i) free radical-generating compounds are tumour promotors, (ii) well-known promotors such as phorbol acetate have been shown to stimulate oxygen radical production, (iii) promotors can modulate antioxidant defense mechanisms and (iv) antioxidants are antipromotors.

Immunomodulatory effects

Several animal studies have shown that carotenoids may exert immunomodulatory functions. For example, T- and B-lymphocytes in the spleens of rats showed enhanced proliferative responses after supplementation with either β-carotene or canthaxanthin [22], whereas hamsters with chemically induced tumours showed increased numbers of cytotoxic T-cells [23]. The postulated immunomodulatory effects of carotenoids have been summarised by Bendich [23]. The mechanisms by which carotenoids may influence immune response are not yet clear. Immuno-enhancing mechanisms have been postulated to involve the quenching of free radicals, which could lower the level of immunosuppressing lipid peroxides, alter arachidonic acid metabolism, stabilise lysozome membranes, or protect nuclear structures. Alternatively, retinol-mediated mechanisms could be involved, since retinol has several influences on the immune system [14]. A possible immunomodulatory effect of carotenoids would imply an action in the promotion phase of carcinogenesis. However, the concept of immunoregulation of carcinogenesis may only apply to certain forms of human cancer, since a substantial number of human cancers do not exhibit any immunogenic properties [24].

Other mechanisms

In addition to the above-mentioned mechanisms other effects of carotenoids have been described. Edes *et al.* [25] observed modulation of the cytochrome P_{450}-dependent aryl hydrocarbon hydroxylase (AHH) in rat liver by β-carotene, whereas Tan and Chu [26] performed *in vitro* experiments that showed modulation of P_{450}-mediated benzo(*a*)pyrene metabolism by carotenoids. These observations suggest that carotenoids could modify the enzymatic activation of (pro)carcinogens. Another mechanism of action has recently been suggested by Zhang *et al.* [27] who observed enhancement of gap–junc communication by several

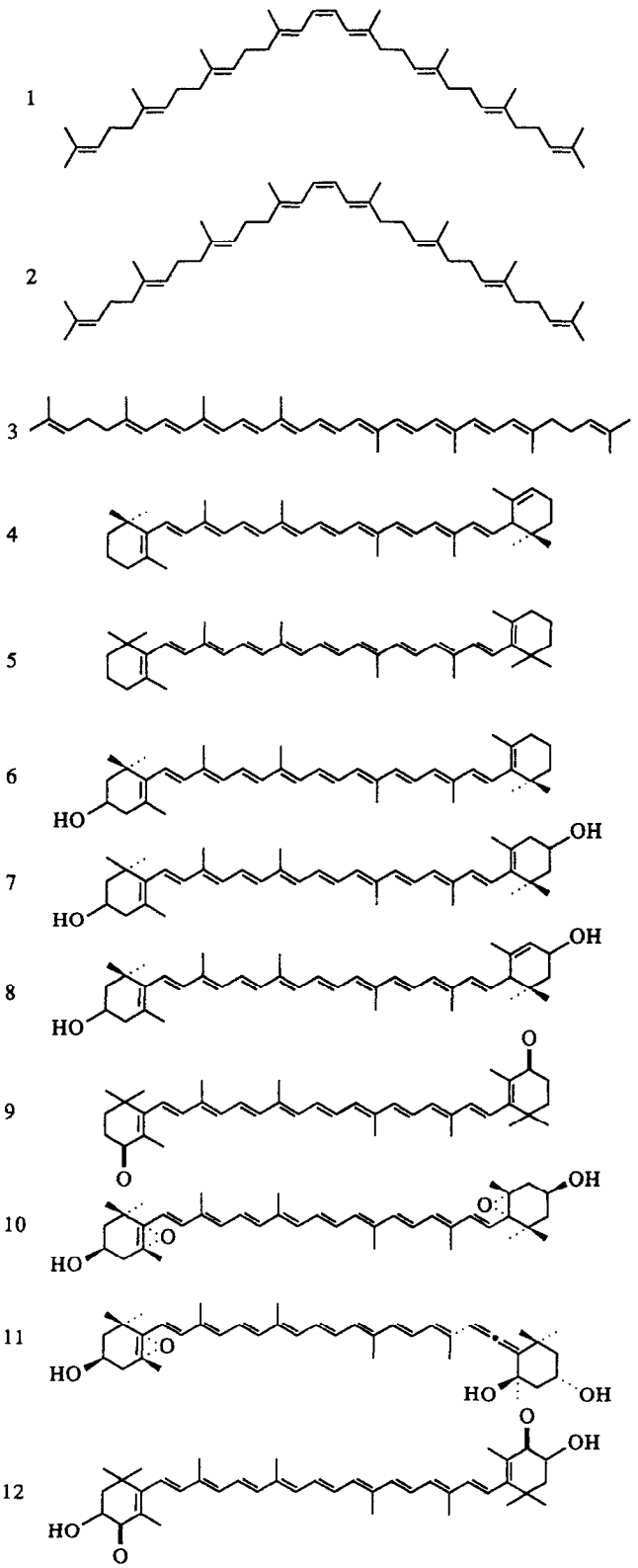

Fig. 2. Polyenes and carotenoids in foods that may also be found in animal tissues. 1, phytoene; 2, phytofluene; 3, lycopene; 4, α-carotene; 5, β-carotene; 6, β-cryptoxanthin; 7, zeaxanthin; 8, lutein; 9, canthaxanthin; 10, violaxanthin; 11, neoxanthin; 12, astaxanthin. (Reproduced with permission from *The FASEB Journal* [6]).

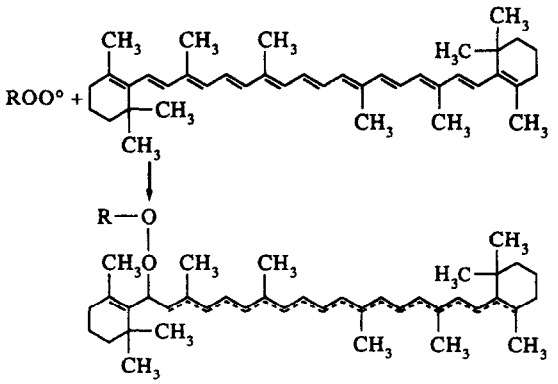

Fig. 3. A proposed interaction between β-carotene and a peroxyl radical to produce a resonance-stabilised carbon-centered radical. (Reproduced with permission from *Science*, Burton *et al.* 1984 [17]. Copyright 1984 by the AAAS).

carotenoids in *in vitro* experiments, irrespective of their pro-vitamin A activity. Enhanced cell-to-cell communication would restrict clonal expansion of initiated cells.

HUMAN INTERVENTION STUDIES

The possible mechanisms of action for carotenoids given above have mainly been derived from *in vitro* experiments. The interest in carotenoids and cancer has also prompted a number of animal studies ([18], for review). Many studies, though certainly not all of them, indicate that carotenoids can prevent the appearance of skin tumours induced by ultraviolet light with or without chemical carcinogens. In addition, carotenoids may also prevent internal tumours induced by carcinogens. The results of these studies are not always consistent, and many workers have used systems unique to their own laboratory. Moreover, the metabolism of carotenoids in most laboratory animals differ noticably from that in humans. Intervention studies in humans are, therefore, necessary to substantiate the evidence from observational epidemiology, *in vitro* experiments and animal studies.

Human intervention studies use either cancer incidence or mortality as an endpoint, or they focus on intermediate endpoints or early biomarkers for cancer risk [28]. These biomarkers are parameters for (alterations in) functions or cellular structures for which the predictive value has not been established but that are thought to bear relevance to carcinogenesis. Such biomarkers include measurements for DNA damage, e.g. micronuclei, measurements of immune system cells or immunological response, or potentially premalignant lesion, e.g. leukoplakia or dysplasia. Human intervention studies that are in progress are listed in Table 3; studies that have already published results are discussed below.

Studies on micronuclei in buccal mucosa of tobacco chewers have been performed by Stich and co-workers. Micronuclei are DNA fragments in exfoliated cells that occur after carcinogenic exposure. Several lines of evidence indicate that they may provide a marker for early stage carcinogenesis, but their predictive value has not been established. Strong reductions in micronucleated buccal mucosa cells were observed after Indian and Fillipino villagers with marginal vitamin A status had been supplemented with β-carotene (180 mg/week), β-carotene combined with retinol (100000 U/week), or retinol alone [29–31]. These results may reflect an enhanced vitamin A intake, and not a specific carotenoid effect since canthaxanthin was not effective [30]. However, it is not clear whether cathaxanthin

*Table 3. Ongoing human intervention trials using β-carotene**

Target site/organ	Target/high risk group	Inhibitory agents	Investigator	Location
All sites	Physicians	β-Carotene	Hennekens	Boston, Massachusetts, U.S.A.
Colon	Colon polyps	β-Carotene	Bowen	Illinois, U.S.A.
Colon	Colon polyps	β-Carotene; vitamins C and E	Greenberg	Hannover, New Hampshire, U.S.A.
Colon	Colon polyps	β-Carotene; Fiber, low fat	MacLennan	Brisbane, Australia
Lung	Asbestosis	β-Carotene; retinol	Omenn	Seattle, Washington, U.S.A.
Lung	Smokers	β-Carotene; vitamin E	Huttunen/ Albanes	Helsinki, Finland. Bethesda, Maryland, U.S.A.
Lung	Tin miners in China	β-Carotene, retinol, vitamin E, Selenium	Schatzkin	Bethesda, Maryland, U.S.A.
Lung	DNA damage, smokers	β-Carotene	Van Poppel	Zeist, Netherlands
Lung	Asbestos workers	β-Carotene, retinol	Musk	Perth, Australia
Skin	Albinos	β-Carotene	Luande	Dar es Salaam, Tanzania
Skin	Basal cell carcinoma	β-Carotene; vitamins C and E	Safai	New York, U.S.A.
Skin	Basal carcinomas	β-Carotene	Siu	Calgary, Canada
Oesophagus	Dysplastic patients and high risk group	Multiple vitamins and β-Carotene	Taylor	Bethesda, Maryland, U.S.A. and Beijing, China
Mouth, oesophagus	Oral leukoplakia and oesophagitis	β-Carotene, retinol, riboflavin	Zaridze	Uzbekistan, former U.S.S.R.
Mouth	Oral leukoplakia	β-Carotene	Garewal	Tuscon, Arizona, U.S.A.
Cervix	Cervical dysplasia	β-Carotene, vitamin C, folic acid	Romney	New York, U.S.A.

* Adapted from: Cullen JW. The National Cancer Institutes Intervention Trials. *Cancer* 1988, 62, 1851–1864.
Coleman M, Wahrendorf J eds. *Directory of On-going Research in Cancer Epidemiology* 1991. IARC scientific publication no. 110. Lyon, International Agency for Research on Cancer, 1991.

does reach the buccal mucosa, as does β-carotene [32] and a subsequent study showed β-carotene also to be effective in snuff users having normal plasma retinol levels [33]. The latter study, however, did not monitor individual plasma retinol or antioxidant levels during the trial.

Immunological studies in humans on the effect of β-carotene so far have focussed on assessment of lymphocyte subpopulations in peripheral blood. These studies must be cautiously interpreted, since the relevance of peripheral lymphocyte subpopulations for functional immune responses in target tissues is not certain. So far, the studies have yielded inconsistent results. Alexander *et al.* [34] reported an increase in the number of lymphocytes expressing CD4+ (indicating T helper function) after 2 weeks of β-carotene (180 mg/day) in normal human volunteers. This study, however, was not placebo controlled, and plasma retinol levels were not monitored. Another study in patients with precancerous lesions [35] used 30 mg/day for 3 months and reported an increase in percentage of lymphocytes expressing Leu-11b (possibly indicating natural killer cell function) and interleukin-2 receptors, whereas the percentage of cells expressing CD4+ was not affected. This study, again, was not placebo controlled and did not monitor retinol levels. Watson *et al.* [36] performed a study in 20 healthy subjects aged 50–65 years using β-carotene doses of 0, 15, 30, 45, and 60 mg/day and report increases in the percentage of lymphoid cells expressing CD4+ and interleukin-2 receptor, as well as an increase in cells expressing CD16+ (indicating natural killer cell function). Watson *et al.*'s study did not observe concomitant changes in plasma retinol, but this study has been methodologically criticised [37]. In contrast to Watson *et al.*'s study, Ringer *et al.* [38] did not observe any effects on lymphocyte subpopulations in a placebo-controlled study in 50 healthy males and females, using doses up to 300 mg/day.

The effect of 6 months β-carotene, with or without retinol, was studied in Indian tobacco chewers showing oral leukoplakia. Leukoplakia is in a potentially premalignant lesion in oral carcinogenesis [31] but the predictive value is not established. As compared with a placebo group, the combination of β-carotene (180 mg/week) and vitamin A (100 000 U/week) resulted in more frequent regression of established leukoplakias, and less frequent appearance of new leukoplakias. β-carotene alone showed some effects, but these were not significant. Here again, the participants may have been vitamin A deficient and plasma retinol levels of the participants were not monitored. Results by Garewal *et al.* [39] indicate (partial) regression of oral leukoplakias in 17 of 24 patients after 3–6 months β-carotene (30 mg/day). However, this pilot study was not placebo-controlled, results may be partly explained by regression to the mean, and plasma retinol levels were not monitored. A randomised, placebo-controlled, 18-month study on oral leukoplakia is now being conducted by the same group [40].

De Vet *et al.* [41] studied the effect of β-carotene (10 mg/day for 3 months) on regression or progression of cervical dysplasia, a putative precursor lesion of cervical carcinoma. Their carefully designed placebo-controlled study showed no effect of β-carotene. Possibly, their dose of β-carotene may have been too low to demonstrate short-term effects. Also, the recently available evidence from observational case–control studies does not indicate a consistent inverse relation between cervical cancer and dietary intake of carotenoids (Table 1). Finally, β-carotene may not affect the late (promotional) stages of carcinogenesis that are reflected by cervical dysplasia.

So far, only one β-carotene intervention study on cancer incidence has been reported [42]. This 5-year placebo-controlled trial showed no effect of β-carotene (50 mg/day) on the occurrence of new skin cancers in persons with previous non-mela-

noma skin cancers. It has been argued, however, that an effect of β-carotene in this study on cancer recurrence could only be expected if β-carotene were effective in very late stages of carcinogenesis [43]. Moreover, the only three observational epidemiological studies performed so far did not show an inverse relation between carotenoids and human skin cancer (Table 2).

CONCLUSIONS AND IMPLICATIONS

A number of human intervention studies on cancer or biomarkers related to cancer risk have been performed so far. These studies have all focussed on β-carotene because this carotenoid has both pro-vitamin A and antioxidant capacity, and has been proven non-toxic [44]. Studies on buccal micronuclei and oral leukoplakia support a cancer preventive role for β-carotene, whereas studies on peripheral immune cells show conflicting results. A study on skin cancer recurrence and a study on cervical dysplasia were negative. It should be realised that the data supportive for β-carotene come from intervention trials on putative markers for cancer risk, rather than trials on cancer incidence. The results nevertheless suggest that β-carotene can affect human carcinogenesis, but also seem to indicate that this will not occur at all stages of carcinogenesis, or at all cancer sites. The ongoing intervention trials (Table 3) will yield more insight into the putative cancer preventive potential of β-carotene. For an unambiguous interpretation, these trials should be methodologically sound, i.e. randomised and placebo controlled. In interpreting these trials, measurements of vitamin A status would be informative as would be measurements of other antioxidants that may act in a synergistic manner. The most promising intervention studies seem to be those that aim at cancer incidence at sites that have been most consistently associated with carotenoid protection. In this respect, two very large ongoing trials on actual cancer incidence are of particular interest. The U.S.A.–Finland lung cancer prevention trial is evaluating the effect of β-carotene (20 mg/day) on lung cancer incidence in 19 500 male smokers [45]. However, the proposed follow-up of 6 years may be insufficient if β-carotene is primarily effective in earlier stages of carcinogenesis. A randomised trial in 22 000 U.S.A. physicians is evaluating the effect of β-carotene (50 mg on alternate days) on cancer at all sites [46]. This trial has recently been extended to 12.5 years follow-up [43]. However, lung cancer incidence may not be very large among predominantly non-smoking physicians.

This view has shown that a high intake of carotenoid-rich fruits and vegetables is associated with a decreased risk of cancer at a number of common sites in epidemiological studies. For several other sites, however, the evidence is not consistent. This association may indeed be due to carotenoids, and not to associated food factors, since a number of plausible cancer preventive mechanisms for carotenoids have been suggested. It is envisaged that the human intervention studies that are currently being conducted will provide more answers regarding the proposed cancer preventive properties particularly of β-carotene.

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Mechanisms of Carcinogenesis: Chemical Exposure and Molecular Changes

Paolo Vineis and Paul W. Brandt-Rauf

THE NUMBER of proposed mechanisms of carcinogenesis is increasing, including indirect mechanisms such as cell proliferation [1–3]. This is of relevance to epidemiology for at least three reasons: (a) the identification of mechanisms of action of human carcinogens sheds light on the biological plausibility of a causal association between an exposure and cancer; (b) some of the most relevant findings on mechanisms have come from investigations in humans, i.e. potentially involving epidemiological expertise; (c) a complete understanding of the cancer process in humans is likely to arise from a combination of conventional epidemiological investigations, aimed at the identification of risk factors, and of biomarker-based studies, aimed at the elucidation of intermediate steps. In fact, cancer epidemiology has been practised until now mainly as a 'black-box' discipline, interested in the (presumed causal) association between an external exposure and the onset of a malignancy. Recently, however, the coupling of epidemiological design with the measurement of biological or biochemical endpoints such as DNA adducts, gene mutations or chromosome aberrations has started to contribute to the unravelling of the intermediate events occurring in the chain between the two extremes of exposure and disease.

Although several carcinogenic mechanisms have been proposed, we will review the epidemiological evidence concerning mutations in proto-oncogenes or tumour suppressor genes, i.e. genotoxic events. Genotoxicity is defined as the capacity of inducing a structural change of genetic material (point mutations; chromosome aberrations), and is particularly relevant to carcinogenesis when proto-oncogenes and tumour suppressor genes are involved. Proto-oncogenes are normal cellular genes that, when *activated* as oncogenes, cause alterations of growth and differentiation, thus enhancing the probability of neoplastic transformation. Tumour suppressor genes are normal cellular genes that, when *inactivated*, also cause alterations of growth and differentiation patterns [4].

Cell proliferation has also been proposed as a mechanism involved in carcinogenesis on the basis of two main considerations: (a) some non-genotoxic chemicals found to be carcinogenic in experimental animals also induce cell proliferation; and (b) an increase in the number of cells which have undergone a first change (e.g. proto-oncogene mutation) (clonal expansion of altered cells) increases the probability of a subsequent carcinogenic 'hit' in a multistep sequence [2]. Cell proliferation would thus increase the probability of cancer by making available a larger number of cells that are vulnerable to subsequent carcinogenic stimuli. Further developments in the theory of cell proliferation in carcinogenesis have invoked, for example, the role of peroxisome proliferation [5] or the accumulation of a particular protein in the rat kidneys [6].

The objective for this paper is to provide a critical review of a few studies involving the measurement of molecular endpoints pertaining to mechanisms of carcinogenesis, and to present an epidemiological point of view on the subject.

CHEMICAL EXPOSURE, GENE MUTATION AND CANCER

The involvement of proto-oncogenes and tumour suppressor genes in chemical carcinogenesis has been repeatedly proposed on the basis of different types of evidence: (a) transfection assays showed that mutated oncogenes were able to transform immortalised cells, i.e. to confer malignant properties; (b) chemical carcinogens are capable of producing mutations in specific loci of proto-oncogenes, for example, mutations in codons 12 and 61 of *ras* are induced by *N*-nitrosocompounds; (c) tumours induced in experimental animals with known carcinogens (*N*-nitrosocompounds, polycyclic aromatic hydrocarbons) showed a high frequency of mutated *ras* oncogenes. Also, epidemiological evidence has been recently published, suggesting the association between chemical exposure, oncogene mutation and cancer onset. The purpose of such studies is not to

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