Carotenoids
Volume 5: Nutrition and Health

Chapter 13

Carotenoids and Cancer

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A. Introduction

Cancer is a major cause of morbidity and mortality around the world, although patterns of cancer, like dietary patterns, are highly variable across regions and countries with different degrees of economic development [1]. Observed associations between dietary patterns and cancer mortality and morbidity have led to hypotheses about cause and effect relationships, which have subsequently been examined more specifically in laboratory studies of biological activities of dietary constituents, case-control and cohort studies within populations, and clinical trials. Food provides nutrients and numerous other bioactive compounds, many of which have been linked specifically to cellular and molecular events and activities that have been identified in the development and progression of cancer [2,3].

Carotenoids are among the bioactive substances that potentially affect risk and progression of cancer, and that have been the focus of numerous investigations. As summarized in comprehensive reviews [4,5], accumulated data on diet and cancer over the past several decades suggest that approximately 30-40% of cancer cases are potentially preventable *via* food choices and the modification of nutritional factors. However, disentangling the effects of various foods, specific dietary constituents, and related lifestyle factors and characteristics (*e.g.* physical activity, obesity) that influence risk and progression of cancer has proved to be very challenging. This challenge is particularly evident when the relationship between carotenoids and cancer is examined, due to the distribution of these compounds in the food supply and the clustering of health-related behaviours. The aim in this *Chapter* is to evaluate the relationship between carotenoids and cancer from data obtained by different experimental approaches. The design, application and interpretation of epidemiology studies are described

in *Chapter 10*. Data on the effects of carotenoids on cellular and molecular processes in cultured cells are presented in *Chapter 11* and the influence of carotenoids on the immune response system is discussed in *Chapter 17*.

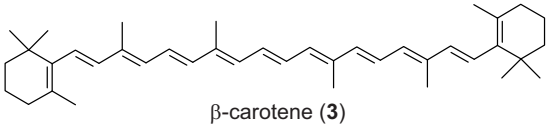
Cancer is one of the leading causes of death worldwide, accounting for 7.6 million (13%) of all deaths, according to World Health Organization 2006 statistics [6]. Over the past 30 years, improvements have been observed in five-year survival rates for all cancers combined and for several specific cancers, and this has been attributed primarily to improved initial treatments and to increased screening that results in diagnosis at an earlier stage. An increasing population of cancer survivors, *i.e*. individuals with a history of cancer who are thus at risk for recurrence or new cancers, has promoted increased interest in whether dietary factors, including carotenoids, may influence this risk and long-term survival [7].

Examining the evidence linking carotenoids to cancer risk and progression requires an appreciation of the multistage process of carcinogenesis. Cancer results from multiple genetic and epigenetic events involving protooncogenes, tumour suppressor genes and antimetastasis genes throughout progression [8]. Specifically, clinical cancer is not determined by a single molecular event that disrupts normal cellular function or regulation of growth but, instead, results from a series of disruptions across the cancer continuum. This continuum extends from the earliest cellular changes, to a preneoplastic lesion, to a malignant tumour, and finally, to metastasis. Genetic or inherited factors play a role in determining susceptibility to molecular and genetic changes in the process of carcinogenesis, although, notably, nutritional factors appear to influence risk even in the presence of highly penetrant, dominant gene mutations [9].

As reviewed previously [10,11], carotenoids have been shown, in laboratory studies, to exhibit several biological activities that could prevent or slow the progression of cancer. In addition to possible antioxidant activity *in vitro* (see *Chapter 12*), carotenoids have favourable effects on cell growth regulation, such as the inhibition of growth and malignant transformation, and the promotion of apoptosis in transformed cells, similar to the effects of retinoids (see *Chapter 11*). However, demonstrating a specific molecular effect of carotenoids in human cancer, in which a series of genetic and epigenetic changes has occurred over years or decades, is logistically challenging.

Interpretation of results of observational studies (see *Chapter 10*) that identify associations between cancer risk and carotenoid intake or concentrations in the circulation is substantially constrained by the risk for confounding, *i.e*. the misidentification of carotenoid intake or plasma concentration (as a biomarker of carotenoid intake and tissue exposure) as the true protective factor instead of other or associated variables. For example, the majority of carotenoids in the diet are contributed by vegetables and fruit. These foods are complex, containing, in addition to carotenoids, numerous constituents that have biological activities [12]. Also, individuals who report consuming higher intakes of vegetables and fruit (and hence carotenoids), or who have higher plasma carotenoid concentrations, typically are more likely to exhibit other prudent health behaviour, such as limited alcohol intake, not smoking, and increased physical activity [13,14].

In general, interpretation of results from observational studies that rely on self-reported dietary data is particularly constrained by the problem of crude and imprecise methods that are used in the collection of these data, as well as a food content database that is of limited quality. The imprecision of assessing status *via* self-reported intakes, as opposed to biomarkers of intake, can result in the wrong conclusions about associations. For example, self-reported intake of vegetables and fruits, the major sources of dietary carotenoids, was not significantly associated with risk for primary breast cancer in the New York University Women's Health Study [15], whereas serum carotenoid concentrations, which are a biomarker for intake of vegetable and fruit intake, were found to be significantly inversely associated with risk in that same cohort of women [16]. The use of suitable dietary biomarkers of carotenoid intake, rather than reliance only on self-reported dietary intake data, is recognized increasingly as being of value for more accurately characterizing usual patterns of intake and true exposure.



β-carotene (**3**)

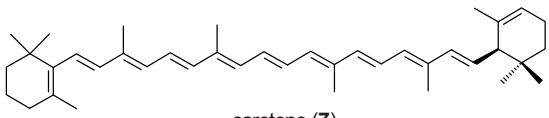
Although randomized clinical trials involving carotenoid supplements would seem to be a preferable approach to testing the specific effect of carotenoids on cancer risk and progression, interpretation of the results of studies reported to date is difficult. Most of these studies have involved individuals who are at very high risk for cancer or who have already been diagnosed with precursor lesions or a primary cancer. For example, trials with supplements of β-carotene (**3**) to test an effect on risk for cervical cancer have all involved women who had already been diagnosed with cervical dysplasia, a precursor neoplastic lesion [17]. If the mechanism by which carotenoids may reduce the risk for cervical cancer, as is suggested by observational epidemiological studies, is a favourable effect on the immune system response to exposure to human papillomavirus (the primary cause of the cancer), it is unlikely that a protective effect of carotenoids would be achieved or observed when the infection is already established. Similarly, β-carotene supplement trials to test the effect of carotenoids on risk for colon cancer have focused on only one stage in the development and progression of this cancer, examining whether β-carotene supplementation, or increased vegetable and fruit intake, over 2-5 years, can affect the risk for adenoma recurrence and growth in individuals with a history of adenomatous polyps [18]. The rationale for using recurrence of colorectal adenomas as the primary end point is that adenomatous polyps are considered precursors of most cancers of the large bowel. A general clinical trial testing the effect of carotenoid intake on incident colon cancer would require a large sample, a very long follow-up period, and considerable resources. However, it must be recognized that, without specific knowledge of the critical points at which carotenoids may affect colon carcinogenesis, the focus of intervention studies

to date may not have tested appropriately the effects of carotenoids on risk for colon cancer. A finding of no effect in a study of this type does not address the possibility that a lifetime of high or low carotenoid intake, or differential carotenoid intakes at another point in the colon cancer continuum, might affect risk for colon cancer.

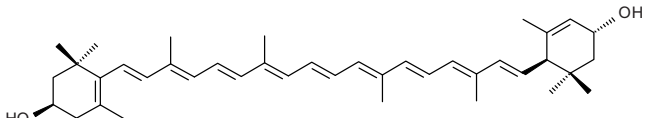
Within these recognized constraints on interpretation and conclusions, available data and current evidence suggest a possible role for carotenoids or at least the major food sources of these compounds, *i.e*. vegetables and fruit, in the aetiology of cancer. The weight of the evidence suggests that carotenoids may influence the risk and progression of the following cancers: lung, breast, prostate, large bowel (colon and rectum), head and neck (oral cavity, pharynx and larynx), cervix and ovary.

B. Lung Cancer

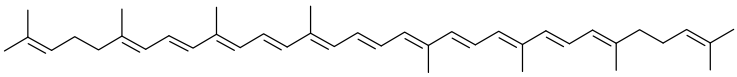
In the U.S. and worldwide, lung cancer is the most common cause of cancer death in both men (31%) and women (26%) and is the second most commonly diagnosed invasive cancer for both gender subgroups (13% and 12% of cases for men and women, respectively) [1,6,7]. Smoking behaviour is an established major environmental factor in the aetiology of lung cancer, but higher intakes of carotenoids and their major food sources, vegetables and fruit, have been associated quite consistently with reduced risk for lung cancer in observational epidemiological studies [19-30]. Although early observational studies mainly revealed an inverse relationship between risk for lung cancer and intakes of total vitamin A (a dietary variable that includes preformed vitamin A as well as provitamin A carotenoids), or of vegetables (especially yellow-orange and dark green vegetables), and higher serum or plasma β-carotene concentration, more recent epidemiological studies have suggested a protective association with other carotenoids. These more recent studies have identified inverse relationships between lung cancer risk and intakes or blood concentrations of α-carotene (**7**), lutein (**133**), lycopene (**31**) and β-cryptoxanthin (**55**), as well as β-carotene.



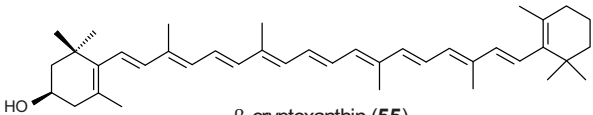
α-carotene (**7**)



lutein (**133**)



lycopene (**31**)



β-cryptoxanthin (**55**)

For example, findings from a multi-centre, case-control study of diet and lung cancer among non-smokers (506 cases, 1,045 controls) found protective effects for intakes of several carotenoids and carotenoid-rich food sources, including tomatoes (odds ratio [OR] 0.5, 95% confidence interval [CI] 0.4, 0.6 for highest *versus* lowest tertile, P = 0.01 for trend), lettuce (OR 0.6, 95% CI 0.3-1.2, P = 0.02 for trend), carrots (OR 0.8, 95% CI 0.5, 1.1, not significant [NS]), total carotenoids (OR 0.8, 95% CI 0.6, 1.0, NS), and β-carotene (OR 0.8, 95% CI 0.6, 1.1, NS) [29]. In another large case-control study (1,000 cases, 1,500 controls) [30], intakes of carrots (relative risk [RR] 0.49, 95% CI 0.31, 0.78 for more than weekly *versus* never), tomato sauce (RR 0.69, 95% CI 0.55, 0.87 for a few times per week or more *versus* never), and tomatoes (RR 0.74, 95% CI 0.57, 0.96 for >45 g/day *versus* <16 g/day) were found to be significantly inversely associated with lung cancer risk.

Note that several ways may be used to express differences between two groups, *e.g*. treated *versus* control. The odds ratio (OR) shows whether the probability of an event is the same in two groups. For example, an OR of 1.0 means that something is equally likely to occur in both groups, an OR of 0.5 means that something is half as likely to occur as it would in a reference group, and an OR of 1.5 means that something is 50% more likely to occur than it would in a reference group. A 95% CI (confidence interval) for a value is the range of values for which one is 95% confident that the true value lies within that range. Relative risk (RR) is the ratio of incidence of disease in the exposed group to incidence of disease in the unexposed group, or the probability of one event divided by the probability of the other event. Hazard ratio (HR) is the ratio of hazard of disease in the exposed group to the hazard of disease in the unexposed or reference group.

In four large placebo-controlled clinical trials, the effect of β-carotene supplementation on lung cancer incidence or mortality was examined [31-34]. Surprisingly, incidence of lung cancer actually increased in response to β-carotene supplementation in two of the studies, particularly among smokers and in subjects who consumed larger amounts of alcohol [35-37]. Results from subsequent studies with laboratory animals suggest that high doses but not low doses (*i.e*. equivalent to 30 mg/day *versus* 6 mg/day in humans) of β-carotene and the resulting cleavage products deplete tissue retinoic acid and interfere with normal retinoid signalling [38,39]. In tissue culture studies, in which the aim is typically to achieve in the cells physiological concentrations, which are considerably lower than the concentrations

achieved in most of the supplement trials, β-carotene has been shown to induce both qualitative and quantitative beneficial changes in lung cancer cells [40]. Clearly, the unregulated uptake by peripheral tissues and demonstrable biological effects on major cell regulatory systems set the stage for potentially adverse, in addition to beneficial, effects of carotenoids on risk for lung cancer.

Integration of current evidence across all types of research suggests that the relationship between carotenoids and lung cancer risk may resemble a bell-shaped curve, with smoking behaviour and alcohol consumption acting as modifiers. At physiological concentrations achieved with doses obtainable from the food supply, carotenoids exhibit biological activities that may be protective against lung cancer; at high concentrations, carotenoids and their cleavage products have adverse effects on cellular function that may be of a magnitude high enough to increase lung cancer risk. It appears that intakes of several carotenoids, and not just β-carotene, may reduce risk for lung cancer, but at doses that would be provided by a diet that includes sufficient amounts of foods that are good sources of these compounds, *i.e*. vegetables and fruit. As noted above, it is unclear at this point whether it is the carotenoids obtained from these foods that are the sole or major protective constituents; laboratory evidence, however, suggests that carotenoids are, at least, among the constituents of these foods that are potentially beneficial in reducing risk for lung cancer.

C. Breast and Ovarian Cancers

Carcinomas of the breast and ovary are hormone-related cancers that have biological similarities. In the U.S., 31% of newly diagnosed invasive cancers are breast cancer and, although five-year survival rates are improving, breast cancer is expected to account for 15% of cancer deaths in women in the U.S. in 2006 [7]. Ovarian cancer is much less common than breast cancer but is more likely to have a worse prognosis. Ovarian cancer accounts for 3% of incident cancers in women in the U.S. but 6% of cancer deaths [7].

1. Breast cancer

As with lung cancer, the vast majority of the early observational studies on diet and breast cancer risk examined intake of β-carotene but not of other carotenoids. Also relevant to associations between risk and carotenoid intake are epidemiological studies of vegetable and fruit intake and breast cancer risk, because a protective effect associated with those foods would indicate that carotenoids might be implicated in the effect. Results from these studies are somewhat supportive but not entirely consistent [1,15,41,42]. In general, results from case-control studies, more than those from cohort studies, suggest that carotenoids may be protective against breast cancer. Few studies have examined the relationship between plasma or serum concentrations of carotenoids and risk for breast cancer. In the largest prospective

study of the relationship between serum carotenoids and risk for breast cancer [16], the odds ratio for the lowest *versus* highest quartile of total serum carotenoids was 2.31 (95% CI 1.35, 3.96). Serum concentrations of β-carotene (OR 2.21, 95% CI 1.29, 3.79), α-carotene (OR 1.99, 95% CI 1.18, 3.34), and lutein (OR 2.08, 95% CI 1.11, 3.90) were all also inversely associated with risk. Other small observational studies that also relied on prediagnostic serum carotenoid concentrations in the analysis of breast cancer risk found some protective associations for serum β-carotene, lycopene, and total carotenoids [43,44], when adjusted for other influencing factors.

Five of the eight epidemiological studies that examined the relationship between intakes of carotenoids, or their major food sources (vegetables and fruit), and survival suggest a possible modest protective effect on prognosis in women who have been diagnosed with breast cancer [45]. Recently, a cohort study involving 1,511 women previously diagnosed and treated for breast cancer, who were followed for an average of seven years, revealed that women in the highest quartile of plasma total carotenoid concentration had an estimated 43% reduction in risk for a new breast cancer event (recurrence or new primary) compared to those in the lowest quintile [46].

Although no clinical trials involving carotenoid supplements and breast cancer risk have been conducted, one recently completed study, the Women's Healthy Eating and Living (WHEL) Study, examined the effect of substantially increased intakes of vegetables and fruits on risk for recurrence and survival, in women who have been diagnosed with breast cancer [47]. In that study, women who were randomly assigned to receive intensive treatment (diet counselling) reported increased carotenoid intakes and exhibited increased plasma concentrations of α-carotene (+223%), β-carotene (+87%), lutein (+29%), and lycopene (+17%) [47], and these levels appear to be fairly well maintained at four-year follow-up. At the end of the study, this was not shown to be associated with reduced risk for additional breast cancer events or mortality during a median 7.3-year follow-up period [48]. However, higher biological exposure to carotenoids, when assessed over the time frame of the study, was associated with greater likelihood of breast cancer-free survival regardless of study group assignment [49].

Compared with the lack of consistency in the epidemiological studies, especially those relying on self-reported dietary data, the laboratory evidence suggesting the feasibility that carotenoids are protective against breast cancer is consistent and convincing. Cell culture studies strongly suggest specific beneficial effects of both provitamin A and non-provitamin A carotenoids on the development and progression of breast cancer [50-52], and the effects are generally retinoid-like effects on cell growth regulation. At this point, the evidence would suggest that carotenoids may reduce risk and progression of breast cancer, although more epidemiological research involving data on serum carotenoid concentrations might better reconcile the observations from human studies with the evidence from cell culture studies.

2. Ovarian cancer

Few epidemiological studies have addressed associations between dietary factors, including carotenoids, and risk for ovarian cancer. Of the case-control studies in which the relationship between risk for ovarian cancer and carotenoids, or their major food sources, vegetables and fruit, have been examined, six studies found protective effects associated with vegetable and fruit intake [53-58] and four studies found protective effects associated with carotenoid intake [54,59-61]. The potential importance of examining intakes over the whole time course of ovarian carcinogenesis is suggested by one large cohort study, in which intake of vegetables and fruit during adolescence, but not intake during adulthood, was found to be protective (RR 0.54, 95% CI 0.29, 1.03 for women who consumed at least 2.5 servings/day *versus* those with lower intakes) [62]. No relationship between serum carotenoid concentrations and risk for ovarian cancer was observed in the sole, very small, prospective study in which serum carotenoids were examined [63]. In one observational study of the effects of various dietary factors on survival in women who had been diagnosed with ovarian cancer, women who reported higher intake of vegetables had significantly greater likelihood of survival than those with lower intake (hazard ratio [HR] 0.75, 95% CI 0.57, 0.99 for highest *versus* lowest tertile) [64].

Clinical trials have not tested whether carotenoid supplementation or dietary modification can influence the risk and progression of ovarian cancer. At this point, data relating ovarian cancer risk to carotenoid intake are limited but suggestive of a possible favourable relationship.

D. Prostate Cancer

Cancer of the prostate is the most commonly diagnosed invasive cancer among men in most developed countries [1]. In the U.S., it accounts for 33% of new cases [7]. Approximately 9% of cancer deaths among men in the U.S. in 2006 may be attributable to prostate cancer. As recently reviewed [65], evidence from the numerous epidemiological studies that have examined whether carotenoids, or foods that are rich sources of these compounds, are associated with risk for prostate cancer has been suggestive but not consistent. Much attention has been focused on the potential protective effect of lycopene or tomato products, the richest source of lycopene in a typical diet in many developed countries. In a meta-analysis of observational studies (11 case-control and 10 cohort studies), high *versus* low intake of tomatoes was associated with an approximate 10-20% reduction in risk for prostate cancer [66]. In general, results from case-control studies have not supported a protective effect of lycopene or tomato products on prostate cancer risk, whereas cohort studies of dietary factors and incident prostate cancer, especially the largest of this type of epidemiological study, have been more supportive [67]. For example, in the Health Professionals Follow-Up Study, in

which dietary intakes and incident prostate cancer were examined in a cohort of 47,365 men, reduced risk of prostate cancer was observed in association with higher lycopene intake (RR 0.84, 95% CI 0.73, 0.96 for high *versus* low quintiles), and a higher level of tomato sauce consumption (RR 0.77, 95% CI 0.66, 0.90 for two or more servings/week *versus* less than one serving/month), controlled for total vegetable and fruit consumption and other influencing factors [68].

Similar to the situation with epidemiological studies of breast cancer, few studies involving serological data indicative of carotenoid status and prostate cancer risk have been reported. The largest of these studies involved blood samples collected from a cohort of 14,916 men, in whom 578 cases were identified, and a statistically significantly lower risk of aggressive prostate cancer (RR 0.56, 95% CI 0.34, 0.92) but not total prostate cancer (RR 0.75, 95% CI 0.54, 1.06) was observed in association with higher plasma lycopene concentrations [69]. Two other smaller cohort studies in which serum lycopene was examined did not identify a protective association [67,70].

Much of the interest in the potential effect of lycopene and food sources of this carotenoid on prostate cancer risk arises from the accumulating laboratory evidence and small clinical studies in which biological markers of prostate cancer have been measured [70]. Because many of these studies tested the effects of tomato products rather than isolated lycopene, it cannot be assumed that the beneficial effects can be attributed to lycopene *per se*. As noted above, studies of the relationship between total vegetable and fruit intake and prostate cancer risk have been inconsistent, as recently reviewed [71,72].

Within one of the placebo-controlled clinical trials testing the effect of β-carotene supplementation on risk for lung cancer, a beneficial effect of β-carotene supplementation on prostate cancer risk was observed [73]. Men in the lowest, *versus* highest, quartile for plasma β-carotene at baseline had a marginally significant (P = 0.07) increased risk for prostate cancer over the trial period. When the men in the lowest quartile for baseline plasma β-carotene concentration were compared based on study group assignment, a significant beneficial effect on risk for prostate cancer was observed in those administrated β-carotene *versus* placebo (RR 0.68, 95% CI 0.46, 0.99). The effect of β-carotene supplementation (50 mg every other day, with or without vitamin C, vitamin E, and a daily multiple vitamin formulation) on prostate cancer will continue to be investigated in 15,000 U.S. men aged 55 years and older in the Physicians' Health Study (PHS) II [74].

Thus, current evidence is suggestive of the possibility that carotenoids may reduce risk for prostate cancer, although more data are clearly needed before the relationship can be considered established. Results from the PHS II Study should provide important insight into the effect of β-carotene supplementation. Although a possible beneficial effect of lycopene on prostate cancer risk remains of great interest, randomized clinical trials testing an effect on a cancer outcome have not been reported. Further, the effects of lycopene specifically, as opposed to a complex food source (tomatoes) are difficult to disentangle in the interpretation of results of epidemiological and many of the laboratory studies that have been reported.

E. Colorectal Cancer

Cancer of the colon is the fourth most commonly diagnosed cancer worldwide, and incidence rates have been increasing steadily, especially in developed countries [1]. In the U.S., colorectal cancer accounts for 10% and 11% of the incident cancer cases in men and women, respectively, and 10% of cancer deaths in both gender subgroups [7]. Results from ecological and migrant studies have long suggested that diet is an important environmental factor that influences the risk and progression of colon cancer. Colon and rectal cancers have a well-established and defined continuum of cellular changes and associated lesions that appear to occur in the stepwise process of developing an invasive tumour.

Numerous observational epidemiological studies have examined associations between intakes of carotenoids or their major food sources, vegetables and fruit, and the risk for colon cancer [1,75]. Intakes of carotenoids and/or vegetables and fruit, and serum concentrations of carotenoids, have been inversely associated with colon cancer risk in the majority of the case-control studies and in studies based on pre-diagnosis serum carotenoid concentrations. Results from the more recent studies, in which intakes of a variety of provitamin A and non-provitamin A carotenoids were examined, have suggested beneficial relationships beyond those previously attributed to β-carotene or vegetable and fruit intake. For example, lutein intake was inversely associated with colon cancer risk in both men and women (OR 0.83, 95% CI 0.66, 1.04 for highest *versus* lowest quintile, P = 0.04 for trend) in a large case-control study (1,993 cases, 2,410 controls), whilst associations with the other carotenoids were not significant [76]. In contrast to results from case-control studies, recent cohort studies have not been as supportive of a protective effect of vegetables and fruit on colon cancer risk [77].

In two large, randomized, controlled trials on the risk for recurrence of adenomatous polyps [78,79], a significant beneficial effect of β-carotene supplementation was not observed. Adenomatous polyps are considered to be preneoplastic lesions, although most adenomas do not progress to carcinomas. The precise time course of a progression, should this occur, is not understood, although clinical evidence suggests that malignancy in an adenoma develops over 20 years or more. The effect of increased intake of vegetables and fruit, aiming for 5-8 servings/day, concurrent with reduced fat intake (20% energy from fat), on adenoma recurrence at four years following randomization was the focus of another large randomized trial, the Polyp Prevention Trial (PPT) [80]. The PPT involved 2,079 study participants, and the absolute difference between the self-reported daily vegetable and fruit intake of the intervention and control groups over the four-year period was 1.1 servings per 1000 kcal/day. No effects on adenoma recurrence were observed. Notably, the intervention group exhibited only a minimal increase (approximately 5%) in serum total carotenoid concentration, despite reporting substantially increased carotenoid intakes in association with reported increased intake of vegetables and fruit. Thus, the PPT did not really test the effects of carotenoids on risk for adenoma recurrence, in view of the minimally increased tissue concentrations that

were achieved. However, results of a secondary analysis of a sub-cohort of PPT study participants (n = 701) are in agreement with prior observational studies: average serum α-carotene, β-carotene, lutein and total carotenoid concentrations at four time points during the study were found to be associated with decreased risk of polyp recurrence (OR 0.71, 0.76, 0.67, and 0.61, respectively, $P < 0.05$) [81].

In relation to the divergent evidence from epidemiological and clinical studies, biological evidence from cell culture studies involving colon cancer cell lines supports the possibility that carotenoids may affect colon cancer risk and progression, and the mechanisms appear to involve both antioxidant and cell growth regulatory activities [82,83]. As noted above, the inherent challenges in collecting and interpreting dietary data in the observational studies, and the limited target and scope of the intervention studies, may explain the different conclusions about the relationship between carotenoids and colorectal cancer risk that can be drawn from the epidemiological, clinical and laboratory findings.

F. Other Cancers

1. Cancer of the oral cavity, pharynx, and larynx (head and neck)

In the U.S., cancers of the oral cavity, larynx and pharynx account for approximately 2% of the incident cancers diagnosed yearly and approximately 1% of cancer deaths [7]. Notably, survival rate remains low for these cancers, compared to other cancers, with an estimated five-year survival rate of 56%.

Results of numerous observational epidemiological studies, including both case-control and cohort studies, quite consistently suggest that the intakes of carotenoids (especially β-carotene), vegetables and fruit are associated with reduced risk for these cancers, as previously reviewed [84]. Further, several studies have tested the effect of β-carotene supplementation on intermediate end points and on selected preneoplastic lesions, such as oral leukoplakia. The majority of these clinical trials showed favourable responses and significantly increased remission rates. In laboratory animal models (rodents), β-carotene has been shown to be protective against oral carcinogenesis [22].

One randomized, placebo-controlled study of the effect of β-carotene supplementation on a head and neck cancer outcome has been conducted and reported. In this study, the target was recurrence and survival rather than primary prevention [85], and 264 men and women with a recent history of head and neck cancer were randomly assigned to receive 50 mg β-carotene/day or placebo and were followed for a median of approximately four years. The intervention had no effect on risk for the primary outcome, which was second primary tumours plus local recurrences (RR 0.90, 95% CI 0.56, 1.45), and no effect on total mortality (RR 0.86, 95% CI 0.52, 1.42). The risks of second head-and-neck cancer and lung cancer were also examined, and no effects on these other outcomes were identified.

Thus, epidemiological evidence, which is fairly consistent, and results from laboratory studies, suggest a potential protective effect of β-carotene on risk for head and neck cancers, with the added support from clinical trials that have focused on intermediate endpoints. However, the effects on primary risk and outcome have been addressed only minimally with a clinical trial that focused only on one aspect of the cancer continuum. Similar to the conclusions regarding carotenoids and colon cancer risk, there is clearly a suggestion of benefit across the range of scientific evidence, but the evidence is inadequate to permit a definite conclusion that carotenoids reduce risk or progression of head and neck cancers.

2. Cervical cancer

Cervical cancer is the third most common cancer among women worldwide [1]. Like colon cancer, invasive cervical cancer is known to arise through a progression of epithelial cell changes across a continuum of lesions classified as cervical intra-epithelial neoplasia (CIN) I, II, III and carcinoma *in situ*, which are earlier stages of this disease. The primary aetiological factor for cervical cancer is known to be human papilloma virus (HPV), although numerous influencing factors, including dietary factors, appear to be among the determinants of whether the HPV persists, disrupts cellular function, and enables progression of disease in the exposed individual.

Studies of dietary intakes of carotenoid-rich foods, or plasma or serum concentrations of carotenoids, linked these compounds inversely to risk and progression of cervical dysplasia quite consistently [86]. In general, evidence for a protective effect on risk for cervical cancer is more consistent in the studies that use serum or plasma carotenoids, compared to case-control observational studies based on self-reported dietary intake of carotenoids. In one small study of the relationship between serum carotenoids and persistence of HPV infection [87], adjusted mean concentrations of serum β-carotene, β-cryptoxanthin, and lutein were, on average, 24% lower ($P < 0.05$) among women who were HPV positive at two time points, compared with those who were HPV negative at both time points or positive at only one time point. In another study that examined this same point in the cervical cancer continuum, higher levels of vegetable consumption were found to be associated with a 54% decreased risk of HPV persistence (adjusted OR 0.46, 95% CI 0.21, 0.97 for highest *versus* lowest tertile, $P = 0.033$ for trend) [88]. Also, plasma concentration of (*Z*)-lycopene was associated with reduced risk for HPV persistence in that study (adjusted OR 0.44, 95% CI 0.19, 1.01 for highest *versus* lowest tertile, $P = 0.046$ for trend).

Five randomized controlled trials to date have tested the effect of β-carotene supplementation on the progression or regression of cervical dysplasia [89]. None of these studies found a beneficial effect compared with placebo. Additionally, a small randomized clinical trial was conducted to test whether consuming a carotenoid-rich diet, high in vegetables and fruit, would promote increased regression of cervical dysplasia in women who had been diagnosed with that preneoplastic lesion. The diet intervention was successful in

promoting increased plasma carotenoid concentrations [90]; however, women assigned to the control group also reported improvements in their diet, and differences in regression rate by study group assignment (diet intervention or control group) were not observed. Overall, consumption of carotenoid-rich foods was associated with increased likelihood of the neoplastic lesion regressing to normal in a year, which is in agreement with the observational studies. Testing the effect of chemoprevention on cervical cancer involving any agent, including carotenoids, is substantially hampered by the facts that spontaneous regression rates are often large and vary a great deal across studies, and that establishing the response, *i.e.* whether regression or progression occurs, in subjects under study is challenging and not readily standardized [89]. The majority of the supplement trials directed towards CIN, as well as the small carotenoid-rich diet intervention study, lacked the statistical power to identify a beneficial effect in this target group at this stage of the cervical cancer continuum.

Cell culture studies have demonstrated that carotenoids can induce growth retardation in cervical dysplasia cell lines and apoptosis in HPV-infected cells [91], thus supporting the evidence from the observational epidemiological studies. Clinical trial results, which have focused solely on the persistence or regression of cervical dysplasia, do not support a protective effect of β-carotene supplementation or a diet high in vegetables and fruit, but the design and limited target of these studies may make these results not applicable to the bigger picture of whether carotenoids affect risk for cervical cancer.

Currently available data suggest that carotenoids may influence the risk and progression of cervical cancer, but increased knowledge of the mechanism would be useful because it would better indicate the time point at which intervention should be directed.

3. Other clinical trials with cancer outcomes

Clinical trials involving β-carotene supplementation have been conducted with a focus on a few additional, although less common, cancer outcomes. Several dietary factors, including β-carotene, have been inversely associated rather consistently with risk for oesophageal and stomach cancer in early epidemiological studies, and two randomized placebo-controlled studies that included β-carotene in a supplement regimen have been conducted in China. In one of these studies, the effect of a supplement that provided 15 mg β-carotene/day plus multivitamins and minerals (*versus* placebo) was tested in 3,318 men and women with oesophageal dysplasia, a precancerous condition [92], and no benefits were observed. In contrast, providing 15 mg β-carotene/day plus vitamin E and selenium was observed to reduce significantly risk for stomach cancer (RR 0.79, 95% CI 0.64, 0.99) in a population-based study conducted in the same region [31]. Results from another randomized controlled trial of β-carotene supplementation, in this case with or without vitamin C, in individuals with a confirmed precancerous gastric lesion (multifocal non-metaplastic atrophy or intestinal metaplasia) suggest improved regression rates in association with β-carotene supplementation, in both conditions [93]. A single randomized trial tested the effect of β-carotene

supplementation (50 mg/day) on recurrent non-melanoma skin cancer over a five-year follow-up period, but significant differences from the placebo group were not observed [94].

As noted above, the interpretation of results of placebo-controlled trials is constrained by the timing in the cancer continuum as well as the follow-up period under study. Nonetheless, the evidence for a protective effect of carotenoids in these other cancers is limited, and an increased knowledge base relating to the core biological issues, such as mechanism, would better inform any future clinical trials.

G. Conclusions

Integrating the findings from the numerous studies that are relevant to defining the relationship between carotenoids and the risk and progression of cancer, across the laboratory and epidemiological studies and clinical trials, involves a critical evaluation of the nature of the data examined in these studies. Although cell-culture studies generally provide strong evidence that these compounds can influence the biochemical, biological and molecular processes involved in the development of cell growth dysregulation and carcinogenesis, these systems are not analogous to the complex intact biological system. Laboratory animal models may not be comparable to the human system in many key biological features that are relevant to carotenoid metabolism and the development of human cancer. Epidemiological studies, especially those based on self-reported dietary data, have numerous constraints and, because they are generally based on carotenoid-rich foods rather than pure carotenoids, attributing beneficial associations and responses to carotenoids may be unwarranted. Even more difficult to interpret in the broader picture of cancer risk and progression are the findings from clinical trials conducted to date. The time frame, target groups and limited scope of these trials have not expanded the knowledge base in many instances, and it is questionable whether the results in most cases are relevant to the long process and multi-factorial nature of human cancer.

The most scientifically supportable conclusion, based on currently available data, is that the weight of the evidence suggests a beneficial effect, although for some cancers more than others, and at doses achievable from the food supply. A diet that includes a sufficient amount of vegetables and fruits, including those that are rich in carotenoids, is a scientifically supportable low-risk strategy that would enable the potential beneficial effects of carotenoids on the risk and progression of cancer to be realized.

References

[1] World Cancer Research Fund/American Institute for Cancer Research, *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research, Washington, D.C. (1997).

[2] J. A. Milner, *J. Nutr*., **134**, 2492S (2004).

[3] P. M. Kris-Etherton, K. D. Hecker, A. Bonanome, S. M. Coval, A. E. Binkowski, K. F. Hilpert, A. E. Griel and T. D. Etherton, *Am. J. Med*., **113**, 71S (2002).
[4] W. C. Willett, *CA Cancer J. Clin*., **49**, 331 (1999).
[5] W. C. Willett, *The Oncologist*, **5**, 393 (2000).
[6] *World Health Organization Fact Sheet No. 297, February 2006*. http://www.who.int/mediacentre/factsheets/fs297/en/print.html
[7] *Cancer Statistics 2006*. American Cancer Society, Atlanta (2006).
[8] C. C. Harris, *Cancer Res*., **51 (suppl.)**, 5023S (1991).
[9] J. Kotsopoulos, O. I. Olopado, P. Ghadrian, J. Lubinski, H. T. Lynch, C. Isaacs, B. Weber, C. Kim-Sing, P. Ainsworth, W. D. Foulkes, A. Eisen, P. Sun and S. A. Narod, *Breast Cancer Res*., **7**, R833 (2005).
[10] N. I. Krinsky, *Ann. NY Acad. Sci*., **854**, 443 (1998).
[11] J. S. Bertram, *Nutr. Rev*., **57**, 182 (1999).
[12] K. A. Steinmetz and J. D. Potter, *J. Am. Diet. Assoc*., **96**, 1027 (1996).
[13] A. K. Kant, *J. Am. Diet. Assoc*., **104**, 615 (2004).
[14] W. E. Brady, J. A. Mares-Perlman, P. Bowen and M. Stacewicz-Sapuntzakis, *J. Nutr*., **126**, 129 (1996).
[15] S. A. Smith-Warner, D. Spiegelman, S. S. Yaun, H. O. Adami, W. L. Beeson, P. A. van den Brandt, A. R. Folsom, G. E. Fraser, J. L. Freudenheim, R. A. Goldbohm, S. Graham, A. B. Miller, J. D. Potter, T. E. Rohan, F. E. Speizer, P. Toniolo, W. C. Willett, A. Wolk, A. Zelniuch-Jacquotte and D. J. Hunter, *JAMA*., **285**, 769 (2001).
[16] P. Toniolo, A. L. Van Kappel, A. Akhmedkhanov, P. Ferrari, I. Kato, R. E. Shore and E. Riboli, *Am. J. Epidemiol*., **153**, 1142 (2001).
[17] C. L. Rock, C. W. Michael, R. K. Reynolds and M. T. Ruffin, *Crit. Rev. Oncol./Hematol*., **33**, 169 (2000).
[18] C. L. Rock, *Recent Results in Cancer Research: Cancer Prevention* (ed. P. M. Schlag and H. J. Senn), **Vol. 174**, p. 171, Springer, Berlin (2007).
[19] R. G. Ziegler, S. T. Mayne and C. A. Swanson, *Cancer Causes Control*, **7**, 157 (1996).
[20] D. Albanes, *Am. J. Clin. Nutr*., **69 (suppl.)**, 1345S (1999).
[21] S. T. Mayne, in *Nutrition in the Prevention and Treatment of Disease* (ed. A. M. Coulston, C. L. Rock and E. R. Monsen), p. 387, Academic Press, San Diego (2001).
[22] International Agency for Research on Cancer. *IARC Handbooks of Cancer Prevention, Vol. 2, Carotenoids*. Oxford University Press, Carey, North Carolina (1998).
[23] L. E. Voorrips, R. A. Goldbohm, H. A. M. Brants, G. A. F. C. Van Poppell, F. Sturmans, J. J. Hermus and P. A. Van den Brandt, *Cancer Epidemiol. Biomarkers Prev*., **9**, 357 (2000).
[24] D. Ratnasinghe, M. R. Forman, J. A. Tangrea, Y. Qiao, S. X. Yao, E. W. Gunter, M. J. Barrett, C. A. Giffen, Y. Erozan, M. S. Tockman and P. R. Taylor, *Alcohol Alcohol.*, **35**, 355 (2000).
[25] D. S. Michaud, D. Feskanich, E. B. Rimm, G. A. Colditz, F. E. Speizer, W. C. Willett and E. Giovannucci, *Am. J. Clin. Nutr*., **72**, 990 (2000).
[26] J. M. Yuan, R. K. Ross, X. D. Chu, Y. T. Gao and M. C. Yu, *Cancer Epidemiol. Biomarkers Prev*., **10**, 767 (2001).
[27] T. E. Rohan, M. Jain, G. R. Howe and A. B. Miller, *Cancer Causes Control*, **13**, 231 (2002).
[28] C. N. Holick, D. S. Michaud, R. Stolzenberg-Solomon, S. T. Mayne, P. Pietinen, P. R. Taylor, J. Virtamo and D. Albanes, *Am. J. Epidemiol*., **156**, 536 (2002).
[29] P. Brennan, C. Fortes, J. Butler, A. Agudo, S. Benhamou, S. Darby, M. Gerken, K. H. Jockel, M. Kreuzer, S. Mallone, F. Nyberg, H. Pohlabeln, G. Ferro and P. Bottetta, *Cancer Causes Control*, **11**, 49 (2000).
[30] S. Darby, E. Whitley, R. Doll, T. Key and P. Silcocks, *Br. J. Cancer*, **84**, 728 (2001).
[31] W. J. Blot, J. Y. Li, P. R. Taylor, W. Guo, S. Dawsey, G. Q. Want, C. S. Yang, S. F. Zheng, M. Gail, G. Y. Li, Y. Yu, B. Liu, J. Tangrea, Y. Sun, F. Liu, J. F. Fraumeni Jr., Y. H. Zhang and B. Li, *J. Natl. Cancer. Inst*., **85**, 1483 (1993).

[32] The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, *New Engl. J. Med.*, **330**, 1029 (1994).
[33] G. S. Omenn, G. E. Goodman, M. D. Thornquist, J. Balmes, M. R. Cullen, A. Glass, J. P. Keogh, F. L. Meyskens, B. Valanis, J. H. Williams, S. Barnhart and S. Hammar, *New Engl. J. Med.*, **334**, 1150 (1996).
[34] C. H. Hennekens, J. E. Buring, J. E. Manson, M. Stampfer, B. Rosner, N. R. Cook, C. Belanger, F. LaMotte, J. M. Gaziano, P. M. Ridker, W. Willett and R. Peto, *New Engl. J. Med.*, **334**, 1145 (1996).
[35] D. Albanes, O. P. Heinonen, P. R. Taylor, J. Virtamo, B. K. Edwards, M. Rautalahti, A. M. Hartman, J. Palmgren, L. S. Freedman, J. Haapakoski, M. J. Barrett, P. Pietinen, N. Malila, E. Tala, K. Liipo, E. R. Salomaa, J. A. Tangrea, L. Teppo, F. B. Askin, E. Taskinen, Y. Erozan, P. Greenwald and J. K. Huttunen, *J. Natl. Cancer Inst.*, **88**, 1560 (1996).
[36] G. S. Omenn, G. E. Goodman, M. D. Thornquist, J. Balmes, M. R. Cullen, A. Glass, J. P. Keogh, F. L. Meyskens, B. Valanis, J. H. Williams, S. Barnhard, M. G. Cherniack, C. A. Brodkin and S. Hammar, *J. Natl. Cancer Inst.*, **88**, 1550 (1996).
[37] N. R. Cook, I. M. Lee, J. E. Manson, J. E. Buring and C. H. Hennekens, *Cancer Causes Control*, **11**, 617 (2000).
[38] X. D. Wang, C. Liu, R. T. Bronson, D. E. Smith, N. I. Krinsky and R. M. Russell, *J. Natl. Cancer Inst.*, **91**, 60 (1999).
[39] R. M. Russell, *Pure Appl. Chem.*, **74**, 1461 (2002).
[40] P. Prakash, T. G. Manfredi, C. L. Jackson and L. E. Gerber, *J. Nutr.*, **132**, 121 (2002).
[41] S. Gandini, H. Merzenich, C. Robertson and P. Boyle, *Eur. J. Cancer*, **36**, 636 (2000).
[42] P. Terry, M. Jain, A. B. Miller, G. R. Howe and T. E. Rohan, *Am. J. Clin. Nutr.*, **76**, 833 (2002).
[43] R. Sato, K. J. Helzlsouer, A. J. Alberg, S. C. Hoffman, E. P. Norkus and G. W. Comstock, *Cancer Epidemiol. Biomarkers Prev.*, **11**, 451 (2002).
[44] S. Ching, D. Ingram, R. Hahnel, J. Belby and E. Rossi, *J. Nutr.*, **132**, 303 (2002).
[45] C. L. Rock and W. Demark-Wahnefried, *J. Clin. Oncol.*, **20**, 3302 (2002).
[46] C. L. Rock, S. W. Flatt, L. Natarajan, C. A. Thomson, W. A. Bardwell, V. A. Newman, K. A. Hollenbach, L. Jones, B. J. Caan and J. P. Pierce, *J. Clin. Oncol.*, **23**, 6631 (2005).
[47] J. P. Pierce, V. A. Newmann, S. W. Flatt, S. Faerber, C. L. Rock, L. Natarjan, B. J. Caan, E. B. Gold, K. A. Hollenbach, L. Wasserman, L. Jones, C. Ritenbaugh, M. L. Stefanick, C. A.Thomson and S. Kealey, *J. Nutr.*, **134**, 452 (2004).
[48] J. P. Pierce, L. Natarajan, B. J. Caan, B. A. Parker, E. R. Greenberg, S. W. Flatt, C. L. Rock, S. Kealey, W. K. Al-Delaimy, W. A. Bardwell, R. W. Carlson, J. A. Emond, S. Faerbeer, E. B. Gold, R. A. Hajek, K. Hollenbach, L. A. Jones, N. Karanja, L. Madlensky, J. Marshall, V. A. Newman, C. Ritenbaugh, C. A. Thomson, L. Wasserman, and M. L. Stefanick, *JAMA*, **298**, 289 (2007).
[49] C. L. Rock, L. Natarajan, M. Pu, C. A. Thomson, S. W. Flatt, B. J. Caan, E. B. Gold, W. K. Al-Delaimy, V. A. Newman, R. A. Hajek, M. L. Stefanick, and J. P. Pierce, *Cancer Epidemiol. Biomarkers Prev.*, **18**, 486 (2009).
[50] V. N. Sumantran, R. Zhang, D. S. Lee and M. S. Wicha, *Cancer Epidemiol. Biomarkers Prev.*, **9**, 257 (2000).
[51] C. L. Rock, R. A. Kusluski, M. M. Galvez and S. P. Ethier, *Nutr. Cancer*, **23**, 319 (1995).
[52] P. Prakash, N. I. Krinsky and R. M. Russell, *Nutr. Rev.*, **58**, 170 (2000).
[53] X. O. Shu, Y. T. Gao, J. M. Yuan, R. G. Ziegler and L. A. Brinton, *Br. J. Cancer*, **59**, 92 (1989).
[54] A. Engle, J. E. Muscat and R.E. Harris, *Nutr. Cancer*, **15**, 239 (1991).
[55] H. A. Risch, M. Jain, L. D. Marrett and G. R. Howe, *J. Natl. Cancer Inst.*, **86**, 1409 (1994).
[56] L. H. Kushi, P. J. Mink, A. R. Folsom, K. E. Anderston, W. Zheng, D. Lazovich and T. A. Sellers, *Am. J. Epidemiol.*, **149**, 21 (1999).
[57] F. Parazzini, L. Chatenoud, V. Chiantera, G. Benzi, M. Surace and C. La Vecchia, *Eur. J. Cancer*, **36**, 520 (2000).

[58] C. Boseti, E. Negri, S. Franceschi, C. Pelucchi, R. Talamini, M. Montella, E. Conti and C. La Vecchia, *Int. J. Cancer*, **93**, 911 (2001).
[59] T. Byers, J. Marshall, S. Graham, C. Mettlin and M. A. Swanson, *J. Natl. Cancer Inst*., **71**, 681 (1983).
[60] M. L. Slattery, K. L. Schuman, D. W. West, T. K. French and L. M. Robison, *Am. J. Epidemiol*., **130**, 497 (1989).
[61] D. W. Cramer, H. Kuper, B. L. Harlow and L. Titus-Ernstoff, *Int. J. Cancer*, **94**, 128 (2001).
[62] K. M. Fairfield, S. E. Hankinson, B. A. Rosner, D. J. Hunter, G. A. Colditz and W. C. Willett, *Cancer*, **92**, 2318 (2001).
[63] K. J. Helzlsouer, A. J. Alberg, E. P. Norkus, J. S. Morris, S. C. Hoffman and G. W. Comstock, *J. Natl. Cancer Inst*., **88**, 32 (1996).
[64] C. M. Nagle, D. M. Purdie, P. M. Webb, A. Green, P. W. Harvey and C. J. Bain, *Int. J. Cancer*, **106**, 264 (2003).
[65] J. M. Chan, P. H. Gann and E. L. Giovannucci, *J. Clin. Oncol*., **23**, 8152 (2005).
[66] M. Etminan, B. Takkouche and F. Caamano-Isorna, *Cancer Epidemiol. Biomarkers Prev*., **13**, 340 (2004).
[67] E. Giovannucci, *Exp. Biol. Med*., **227**, 852 (2002).
[68] E. Giovannucci, E. B. Rimm, Y. Liu, M. J. Stampfer and W. C. Willett, *J. Natl. Cancer. Inst*., **94**, 391 (2002).
[69] P. H. Gann, J. Ma, E. Giovannucci, W. Willett, F. M. Sacks, C. H. Hennekens and M. J. Stampfer, *Cancer Res*., **59**, 1225 (1999).
[70] C. W. Hadley, E. C. Miller, S. J. Schwartz and S. K. Clinton, *Exp. Biol. Med*., **227**, 869 (2002).
[71] D. Albanes and T. J. Hartman, in *Diet, Nutrition, and Health* (ed. A. M. Papas), p. 497, CRC Press, Boca Raton (1999).
[72] A. R. Kristal and J. H. Cohen, *Am. J. Epidemiol*., **151**, 124 (2000).
[73] N. R. Cook, M. J. Stampfer, J. Ma, J. E. Manson, F. M. Sacks, J. E. Buring and C. H. Hennekens, *Cancer*, **86**, 1783 (1999).
[74] W. G. Christen, J. M. Gaziano and C. H. Hennekens, *Ann. Epidemiol*., **10**, 125 (2000).
[75] J. D. Potter, *Cancer Causes Control*, **7**, 127 (1996).
[76] M. L. Slattery, J. Benson, K. Curtin, K. N. Ma, D. Schaeffer and J. D. Potter, *Am. J. Clin. Nutr*., **71**, 575 (2000).
[77] K. B. Michels, E. Giovannucci, K. J. Joshipura, B. A. Rosner, M. J. Stampfer, C. S. Fuchs, G. A. Colditz, F. E. Speizer and W. C. Willett, *J. Natl. Cancer Inst*., **92**, 1740 (2000).
[78] E. R. Greenberg, J. A. Baron, T. D. Tosteson, D. H. Freeman, G. J. Beck, J. H. Bond, T. A. Colacchio, J. A. Coller, H. D. Frankl, R. W. Haile, J. S. Mandel, D. W. Nierenberg, R. Rothstein, D. C. Snover, M. M. Stevens, R. W. Summers and R. W. Van Stock, *New Engl. J. Med*., **331**, 141 (1994).
[79] R. MacLennan, F. Macrae, C. Bain, D. Battistutta, P. Cahpuis, H. Gratten, J. Lambert, R. C. Newland, M. Ngu, A. Russell, M. Ward and M. L. Wahlqvist, *J. Natl. Cancer Inst*., **87**, 1760 (1995).
[80] A. Schatzkin, E. Lanza, D. Corle, P. Lance, F. Iber, B. Caan, M. Shike, J. Weissfeld, R. Burt, M. R. Cooper, J. W. Kikendall and J. Cahill, *New Engl. J. Med*., **342**, 1149 (2000).
[81] S. Steck-Scott, E. Lanza, M. Forman, A. Sowell, C. Borkowf, P. Albert and A. Schatzkin, *FASEB J*., **15**, A62 (2001).
[82] P. Palozza, S. Serini, N. Maggiano, M. Angelini, A. Boninsegna, F. Di Nicuolo, F. R. Ranelletti and G. Calviello, *Carcinogenesis*, **23**, 11 (2002).
[83] P. Palozza, G. Calviello, S. Serini, N. Maggiano, P. Lanza, F. O. Ranelletti and G. M. Bartola, *Free Radic. Biol. Med*., **30**, 1000 (2001).
[84] S. T. Mayne and S. M. Lippman, in *Principles and Practice of Oncology, 6th Edition* (ed. V. T. DeVita, S. Hellman and S. A. Rosenberg), p. 575. Lippincott Williams and Wilkins, Philadelphia (2001).

[85] S. T. Mayne, B. Cartmel, M. Baum, G. Shor-Posner, B. G. Fallon, K. Briskin, J. Bean, T. Zheng, D. Cooper, C. Friedman and W. J. Goodwin, *Cancer Res*., **61**, 1457 (2001).
[86] N. Potischman and L. A. Brinton, *Cancer Causes Control*, **7**, 113 (1996).
[87] A. R. Giuliano, M. Papenfuss, M. Nour, L. M. Canfield, A. Schneider and K. Hatch, *Cancer Epidemiol. Biomarkers Prev*., **6**, 917 (1997).
[88] R. L. Sedjo, D. J. Roe, M. Abrahamsen, R. B. Harris, N. Craft, S. Baldwin and A. R. Giuliano, *Cancer Epidemiol. Biomarkers Prev*., **11**, 876 (2002).
[89] M. Follen, F. L. Meyskens, N. Atkinson and D. Schotttenfeld, *J. Natl. Cancer Inst*., **93**, 1293 (2001).
[90] C. L. Rock, A. Moskowitz, B. Huizar, C. C. Saenz, J. T. Clark, T. L. Daly, H. Chin, C. Behling and M. T. Ruffin, *J. Am. Diet. Assoc*., **101**, 1167 (2001).
[91] Y. Muto, J. Fujii, Y. Shidoji, H. Moriwaki, T. Kawaguchi and T. Noda, *Am. J. Clin. Nutr*., **62(suppl)**, 1535S (1995).
[92] J. Y. Li, P. R. Taylor, B. Li, S. Dawsey, G. Q. Wang, A. G. Ershow, W. Guo, S. F. Liu, C. S. Yang and Q. Shen, *J. Natl. Cancer Inst*., **85**, 1492 (1993).
[93] P. Correa, E. T. H. Fontham, J. C. Bravo, L. E. Bravo, B. Ruiz, G. Zarama, J. L. Realpe, G. T. Malcom, D. Li, W. D. Jonson and R. Mera, *J. Natl. Cancer Inst*., **92**, 1881 (2000).
[94] E. R. Greenberg, J. A. Baron, T. A. Stukel, M. M. Stevens, J. S. Mandel, S. K. Spencer, P. M. Elias, N. Lowe, D. W. Nierenberg, G. Bayrd, J. C. Vance, D. H. Freeman, W. E. Clendenning and T. Kwan, *New Engl. J. Med*., **323**, 789 (1990).