Antioxidants and the Prevention of Cancer

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Introduction

Oxidative damage to DNA, lipids and proteins in the human body is generally considered to be an important factor in carcinogenesis. Reactive oxygen species such as superoxide, nitric oxide and hydroxyl radicals, formed continuously as a result of biochemical reactions, can cause significant oxidative damage. Also, environmental carcinogens from various sources – for instance, tobacco smoke and industrial pollution – and food contaminants such as heterocyclic aromatic amines (HAAs) contribute to an individual's total burden of oxidative stress (Jacob and Burri, 1996; Loft and Poulsen, 1996). Antioxidant defences – for example, enzymes that continually repair DNA damage – frequently cannot counteract all of the oxidative attack, and the resulting damage may lead to genetic mutations that could contribute to carcinogenesis. Dietary antioxidants, ubiquitous in plant foods where they have evolved to protect the plants against oxidative assault, may be protective to humans, in terms of reducing cancer risk. Reactive oxygen species appear to be involved at all stages of cancer development; consequently, dietary antioxidants may have potential benefits throughout the carcinogenic process (Diplock, 1996; Cozzi *et al*., 1997).

Epidemiologic data provide strong evidence of a cancer-protective effect for high intakes of vegetables, fruits and whole grains. Many studies indicate that the risk of cancer incidence associated with lowest vegetable and fruit intakes is approximately twice that associated with highest intakes (Block *et al*., 1992). A review of more than 200 case–control and cohort studies found a probable protective effect for cancers of the breast, colon,

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endometrium, oral cavity and pharynx, pancreas and bladder, and convincing evidence for inverse associations with cancers of the stomach, oesophagus, and lung (Steinmetz and Potter, 1996). In 85% of the studies, raw vegetables appeared to be protective; highly protective categories included *Allium* vegetables, carrots, green vegetables, cruciferous vegetables, and tomatoes – all protective in 70% or more of the studies. Total fruits and citrus fruit were protective in about 65% of the studies. The cancer-inhibitory effects reported for these plant foods may be attributed in part to various antioxidant constituents, including micronutrients (e.g. β-carotene (provitamin A), vitamins E and C, selenium) and certain phytochemicals (e.g. polyphenolics, carotenoids) (Wattenberg, 1992). It is likely that numerous constituents contribute to the overall protective effect. Lung cancer risk, for instance, consistently has been shown to be reduced by high vegetable and/or fruit consumption in both prospective and retrospective epidemiological studies, but the beneficial effect cannot be explained completely by either β-carotene or total provitamin A carotenoid intakes (Ziegler *et al.*, 1996a).

When interpreting the results of epidemiological studies, it must be kept in mind that the effects of dietary factors on cancer risk can be influenced by an individual's genetic susceptibility. Several of the more common variations in susceptibility among individuals result from polymorphisms in specific genes (including *CYP1A1*, *CPY1A2*, *CYP2D6*, *GSTM1* and *NAT2*) that cause differences in metabolic or detoxification activities (Perera, 1996). Increased risk of lung cancer, for example, has been correlated with variant forms of *CYP1A1* (catalyses the oxygenation of polyaromatic hydrocarbons (PAHs) and is induced by cigarette smoke), *CYP2D6* (acts on an unknown substrate, possibly tobacco-specific nitrosamines), and *GSTM1* (detoxifies reactive, electrophilic compounds such as PAHs) (Perera, 1996). The relative prevalence and/or distribution of such polymorphisms in populations targeted in epidemiological studies may affect individual responses to both risk and protective factors, including antioxidants and other dietary constituents, and thus may contribute to confounding in analysis and interpretation of study results.

The Chemoprevention Program developed at the National Cancer Institute (NCI) is systematically carrying out preclinical and clinical studies on numerous potential agents identified by surveying a broad spectrum of epidemiological, laboratory and clinical research for compounds that have demonstrated apparent cancer-protective activity. Since the programme's inception in 1987, more than 400 agents have been entered and more than 250 of these have been tested in animal screens. Based on Phase I pharmacokinetic and clinical safety trials, the most promising agents progress to Phase II and III clinical trials; more than 30 agents, including naturally occurring antioxidant micronutrients and phytochemicals, as well as synthetic antioxidants, are currently being studied.

Micronutrients

β-Carotene

Clinical chemoprevention trials using β-carotene are based on extensive evidence from epidemiological studies. Weighing the evidence from studies conducted in the 1970s, which linked high vegetable and fruit intake – an index of β-carotene intake – with reduced cancer risk, Peto and colleagues (1981) suggested that β-carotene might be the protective factor in these foods. Several possible mechanisms were proposed that supported the biological plausibility of β-carotene as a cancer-protective agent, including antioxidant protection and the differentiating action of vitamin A formed from β-carotene. The encouraging epidemiological evidence, as well as supporting laboratory data, provided a strong scientific rationale for the hypothesis that β-carotene can reduce cancer risk. Randomized, controlled clinical chemoprevention trials, the only definitive way to test such hypotheses, were initiated in the 1980s; results of these trials are described below in the section 'Large-scale, Randomized Intervention Trials'.

Early clinical trials on oral cancer showed evidence of benefit from β-carotene. In Filipino betel nut chewers, who are at very high risk for oral cancer, the percentage of buccal mucosa cells with micronuclei – evidence of genotoxic damage – was significantly lower in people given β-carotene for 9 weeks than in those given canthaxanthin, a carotenoid with no vitamin A activity (Stich *et al.*, 1984). One study that examined the effect of β-carotene on leukoplakia, a precancerous oral lesion, reported that 17 of 24 patients showed significant reversal of lesions after 6 months of treatment (Garewal *et al.*, 1990). In another study, however, low-dose 13-*cis*-retinoic acid was significantly more effective than β-carotene in maintaining the stability of leukoplakia reversed by high-dose 13-*cis*-retinoic acid (Lippman *et al.*, 1993).

Trials to prevent colorectal polyps showed no evidence of benefit from β-carotene. In a study in which 864 people, who previously had polyps removed, received either β-carotene, vitamins C and E, all three antioxidants, or placebo for 4 years, no reduction in polyp incidence – and no evidence of harm – was demonstrated for any of the interventions (Greenberg *et al.*, 1994). Similarly, an Australian polyp prevention trial, in which approximately 400 patients with previous polyps received either β-carotene or placebo along with usual diet, low-fat diet, high-wheat bran diet or low-fat/high-wheat bran diet, found no significant polyp reduction (MacClennan *et al.*, 1995). The data suggested, however, that the low-fat/high-wheat bran diet may inhibit the transition from smaller to larger polyps, which have greater malignant potential.

Numerous epidemiological studies investigating the possible association between β-carotene and the risk of various types of cancer have been conducted since the early 1980s, concurrent with clinical trials. A significant protective effect of dietary β-carotene for lung cancer is strongly supported

by epidemiological data. A review by van Poppel and Goldbohm (1995) of approximately 80 retrospective studies and 50 prospective studies reported that associations of either high intakes of β-carotene-rich vegetables and fruits or high blood concentrations of β-carotene with reduced cancer risk were most consistent for lung and stomach cancer. Oesophageal cancer also showed a promising risk reduction, but the number of studies was limited. Reported results for the effects of both β-carotene and vitamin A on prostate cancer have been equivocal, with studies being almost equally divided between inverse and positive associations (Kolonel, 1996). For breast cancer, evidence from case–control studies has indicated a possible protective effect of β-carotene (Hunter and Willett, 1996). For colon cancer, some case–control studies have reported significantly reduced risk at high intakes, but the overall data have suggested only a modest risk reduction (van Poppel and Goldbohm, 1995).

Vitamin E/vitamin C

Vitamin E

Epidemiological studies that have investigated associations of cancer risk at specific sites and diets high in vitamin E (α-tocopherol) are limited in number and data are inconsistent, possibly because estimation of vitamin E intake is difficult (Byers and Guerrero, 1995; Steinmetz and Potter, 1996). A review that examined case–control and cohort studies of vitamin E intake and serum vitamin E levels in relation to breast cancer risk reported that the data were inconclusive (Kimmick *et al*., 1997). One case–control study that investigated the association between micronutrient intake and colorectal adenomas reported that men in the highest quartile of vitamin E intake, compared with the lowest quartile, were about one-fifth as likely to develop adenomas (OR=0.22 for men and 0.74 for women), after adjustment for possible confounding variables, including other micronutrients (Tseng *et al*., 1996). Similarly, another case–control study showed decreased risk of adenoma for the highest tertile of vitamin E intake (OR=0.6, men and women combined, adjusted for total energy and physical activity) (Lubin *et al*., 1997). In the recently completed Alpha-Tocopherol Beta-Carotene (ATBC) Cancer Prevention Study, conducted in Finland in more than 29,000 male cigarette smokers at high risk for lung cancer, 34% fewer cases of prostate cancer and 16% fewer cases of colorectal cancer were diagnosed among men who received daily vitamin E supplements (ATBC Cancer Prevention Study Group, 1994). Although these results suggest a protective effect of vitamin E, prostate and colon cancers were not primary study endpoints. Further research, targeted at these cancers, is needed before conclusions can be drawn about the potential of vitamin E for their prevention. A recent review suggests that antioxidant effects of γ-tocopherol may be superior to those of α-tocopherol, and that studies of vitamin E and colorectal cancer should consider all tocopherols (Stone and Papas, 1997).

Vitamin C

Epidemiological evidence for a protective effect of diets high in vegetables and fruits that contain vitamin C is strong and consistent for cancers of the oral cavity, oesophagus and stomach, but moderate and less consistent for colon and lung cancers. The data do not support an association with prostate cancer and the evidence for breast cancer has been conflicting (Block, 1991; Byers and Guerrero, 1995). A review of more than 50 case–control and cohort studies that investigated intakes of vegetables and fruits, vitamin C, and vitamin E, reported that, across studies, individuals in the highest quartile or quintile of vegetable and fruit intake had approximately 40% less risk of gastrointestinal and respiratory tract cancers than those in the lowest intake levels (Byers and Guerrero, 1995). Indices of vitamin C computed from vegetable and fruit intakes also were associated with lower risk.

Selenium

The trace mineral selenium, although not an antioxidant *per se*, functions as a cofactor for glutathione peroxidase, an enzyme that may protect against oxidative tissue damage (Steinmetz and Potter, 1996). Dietary assessment of selenium can be difficult because the selenium content of plant foods depends on the selenium content of the soil in which the plants grow. Thus, selenium status is best determined by biochemical measures, such as blood or toenail selenium levels. Cancer mortality international correlation studies suggest an inverse association between selenium status and cancer incidence (Schrauzer *et al*., 1977). Also, animal studies strongly support a cancer-protective effect of selenium (Kelloff and Boone, 1996a). Data from most case–control and cohort studies, however, have not been convincing for those cancer sites investigated, including lung, breast and stomach cancers (Hunter and Willett, 1996; Kono and Hirohata, 1996; Ziegler *et al*., 1996a). Data from a recent prospective study that examined the association between serum selenium levels and development of ovarian cancer indicated that women in the highest tertile, compared with the lowest tertile, were four times less likely to develop ovarian cancer (OR=0.23) (Helzlsouer *et al*., 1996). A randomized, controlled clinical intervention trial that tested whether a daily 200 μg selenium supplement will decrease incidence of basal cell and squamous cell skin cancers found no protective effect against skin cancer. Secondary endpoint analyses, however, showed significant reductions in total cancer mortality (RR=0.5), total cancer incidence (RR=0.63), and incidences of lung (RR=0.54), colorectal (RR=0.42) and prostate (RR=0.37) cancers, for individuals who received selenium supplements, compared with controls (Clark *et al*., 1996). These findings support the cancer-protective effect of selenium, but must be confirmed in independent intervention trials.

The effective doses and toxic doses of organoselenium compounds, such as selenomethionine and selenocysteine, are quite close. Synthesis of

1,4-phenylenebis(methylene)-selenocyanate (*p*-XSC) and benzylselenocyanate (BSC) has provided effective, less toxic alternatives. Preclinical data indicate that administration of either *p*-XSC or *p*-methoxy-BSC during the postinitiation phase significantly reduces the formation of chemically induced colon tumours in rats fed a high-fat diet (Reddy *et al*., 1997). In this study, the chemopreventive effects of *p*-XSC were enhanced in animals fed a low-fat diet. The NCI is currently sponsoring preclinical toxicity studies for *p*-XSC; if results are acceptable, a Phase I clinical trial will be considered (Kelloff and Boone, 1996a). Determining the optimal form and dose level of selenium for use in clinical trials, however, is complicated by the fact that toxicity thresholds may differ among individuals.

Phytochemicals

Vegetables, fruits and whole grains contain a wide variety of primarily non-nutritive phytochemical antioxidants, such as phenolic/polyphenolic compounds and carotenoids, that have the potential to modulate cancer development (Wattenberg, 1992). To illustrate, the oil obtained from the first cold pressing of olives can contain as much as 1 g kg^{-1} of polyphenolic compounds with antioxidant potential, including oleuropein (the principal bitter constituent in olives), (3,4-dihydroxyphenyl)ethanol and (*p*-hydroxyphenyl)ethanol (Visioli *et al*., 1995). In an experimental model, (3,4-dihydroxyphenyl)ethanol demonstrated a protective effect on Caco-2 human colon cancer cells subjected to chemically induced oxidative stress, suggesting that dietary intake of olive oil polyphenols could protect against damage by reactive oxygen species (Manna *et al*., 1997).

The NCI has conducted preclinical screening on approximately 100 potential chemopreventive agents that are classified as antioxidants, many of which are naturally occurring, such as fumaric acid, quercetin, (+)-catechin, chlorogenic acid, ellagic acid, ascorbyl palmitate, ethylvanillin, 18-glycyrrhetinic acid and vitamin E acetate (Steele *et al*., 1996). Several antioxidant phytochemicals that have shown promise in screening and/or epidemiological studies have progressed to early-stage clinical testing. Selected antioxidant phytochemicals that may be beneficial in reducing cancer risk are highlighted below.

Phenolic compounds

Tea extracts/green tea polyphenols (GTP)

Although the data are not wholly consistent, epidemiological studies have suggested a protective effect of black (oxidized) or green (unoxidized) tea consumption against cancers of the breast, colon and rectum, lung, nasopharynx, stomach, uterus, gall bladder, liver and pancreas (Stoner and

Mukhtar, 1995; Yang *et al*., 1996). For instance, a case–control study in Shanghai, China, recently reported an inverse association for green tea consumption (highest tertile vs. lowest tertile) and cancer risk for colon (men, RR=0.82; women, RR=0.67), rectum (men, RR=0.72; women, RR=0.57), and pancreas (men, RR=0.63; women, RR=0.53), with statistically significant trends for both rectal and pancreatic cancers (Ji *et al*., 1997). Also, a prospective cohort study in Japan found that consumption of more than ten cups of green tea daily reduced cancer risk among both women (RR = 0.57) and men (RR = 0.68), after adjustment for confounding variables (Imai *et al*., 1997). A significant delay of cancer onset, about 9 years, was found in women who consumed more than ten cups daily vs. those who consumed less than three cups daily; for men and women combined, this delay, also significant, was 4 years. Most experimental studies examining a possible relationship between tea and cancer risk have focused on extracts of either black or green tea and on GTP, including (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG). EGCG, the major catechin in green tea, accounts for 40% of GTP and possesses the greatest anticarcinogenic activity (Stoner and Mukhtar, 1995). Findings in one recent study suggest that GTP may protect against cancer by causing cell cycle arrest and inducing apoptosis (Ahmad *et al*., 1997).

Curcumin

Curcumin, the yellow colouring agent in the spice turmeric, has effectively inhibited chemically induced tumours in the skin, forestomach, mammary gland (*in vitro*), colon (*in vitro*) and duodenum of rodents (Kelloff *et al*., 1994). In combination studies, curcumin plus *N*-(4-hydroxyphenyl)retinamide (4-HPR) appeared to be more effective than either agent alone against rat mammary carcinogenesis (Kelloff and Boone, 1996b). The NCI is sponsoring a Phase I trial of curcumin in normal, healthy subjects to define both its safety and pharmacokinetic characteristics. A Phase II trial of curcumin in dysplastic oral leukoplakia patients, using topical administration, also is being considered.

Ellagic acid

Ellagic acid, found in certain berries and nuts, has exhibited anticarcinogenic effects for chemically induced tumours of the lung, skin, oesophagus and liver in experimental studies (Kelloff *et al*., 1994; Stoner and Mukhtar, 1995). Pharmacokinetic studies with ellagic acid, however, suggest that its poor bioavailability may adversely affect usefulness in humans (Kelloff *et al*., 1994).

Carotenoids

Common green, yellow/red and yellow/orange vegetables and fruits contain more than 40 carotenoids, in addition to β-carotene, that can be absorbed and metabolized by humans, including lutein, zeaxanthin, cryptoxanthin, lycopene, α-carotene, phytofluene, phytoene, astaxanthin, canthaxanthin and crocetin (Khachik *et al*., 1995). As a class, carotenoids exhibit strong antioxidant activity, increase metabolic detoxification, increase cellular communication and have anti-inflammatory properties (Kelloff and Boone, 1994). Preliminary human metabolic studies on lutein and lycopene, two major dietary carotenoids, and zeaxanthin, a dihydroxycarotenoid isomeric to lutein, have established that these compounds undergo oxidation *in vivo*, clearly demonstrating their antioxidant capabilities (Khachik *et al*., 1995).

Using a carotenoid database compiled for more than 2400 vegetables, fruits and multicomponent foods containing vegetables and fruits (Chug-Ahuja *et al*., 1993), Ziegler and colleagues (1996b) reanalysed data from a population-based study conducted in New Jersey during 1980 and 1981 to evaluate the hypothesis that β-carotene may be protective against lung cancer. The reanalysis estimated smoking-adjusted risk of lung cancer in white male current and recent cigarette smokers by intakes of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, α-carotene + β-carotene + lutein/zeaxanthin and total carotenoids. Men in the lowest quartile of α-carotene intake had more than twice the risk of men in the highest quartile. The corresponding risks associated with intakes of β-carotene and lutein/zeaxanthin were increased only about 60%, suggesting that β-carotene is not the dominant protective factor in vegetables and fruits for lung cancer (Ziegler *et al*., 1996b). Ecological data suggested that an inverse association with lutein (but not with α- or β-carotene) accounted for 14% of the variation in incidence in Fiji, where lung cancer rates are markedly lower than for other South Pacific islands (Le Marchand *et al*., 1995). Data from a nested case–control study of serum micronutrient levels (highest vs. lowest tertiles) in a cohort of Japanese–American men indicated that α-carotene (RR = 0.19), β-carotene (RR = 0.10), β-cryptoxanthin (RR = 0.25) and total carotenoids (RR = 0.22) all significantly reduce the risk of aerodigestive tract cancers (Nomura *et al*., 1997a).

Carotenoids also have been associated with reduced risk for hormone-related cancers. In a case–control study in Italian women, decreased breast cancer risk was associated with dietary intakes of α-carotene (RR=0.58, highest quintile) and β-carotene (RR = 0.68, highest quintile); however, when both α-carotene and β-carotene were introduced into the same model, only α-carotene remained protective. Intakes of β-cryptoxanthin, lycopene, and lutein/zeaxanthin were not related to risk (La Vecchia *et al*., 1998). Although numerous epidemiological studies have reported inverse associations between either dietary total carotenoids, β-carotene, or other

specific carotenoids and cervical cancer risk (Potischman and Brinton, 1996), results from β-carotene chemoprevention trials have not been promising, possibly in part because small sample sizes limited the power of the studies (Giuliano and Gapstur, 1998).

One review of epidemiological studies of vegetable and fruit intake and cancer risk suggested a slightly increased risk of prostate cancer among those with low intake (RR=1.3) (Block *et al.*, 1992). A later review reported inconsistent results for prostate cancer; six prospective studies failed to confirm the inverse association found in three out of five case–control studies (van Poppel and Goldbohm, 1995). Results from one large prospective epidemiological cohort study suggested that significant reduction in prostate cancer may be associated with increased dietary intake levels of lycopene (RR=0.79, highest vs. lowest quintile) and tomato-based foods (RR=0.65), which are high in lycopene (Giovannucci *et al.*, 1995). Results from a study of serum carotenoids in a cohort of American men of Japanese descent, however, did not support a reduced prostate cancer risk for any carotenoid, including lycopene (Nomura *et al.*, 1997b). Analysis of prostate tissue has determined that lycopene is present in biologically active concentrations, supporting the hypothesis that lycopene could have direct cancer-protective effects within the prostate (Clinton *et al.*, 1996).

Large-scale, Randomized Intervention Trials

The randomized, controlled clinical intervention trial is considered the best means available to determine whether antioxidants actually do reduce cancer risk. Selected ongoing and completed NCI-sponsored chemoprevention trials using antioxidants are described briefly below. The laboratory data and epidemiological studies that linked high intakes of β-carotene-containing foods and high blood levels of β-carotene to reduced risk of lung cancer provided strong hypotheses for the interventions used in these chemoprevention trials. Although the results were not as expected, these studies demonstrate the difficulty of isolating a single component of a healthful diet as the one beneficial element and exemplify the need for large-scale clinical trials.

Ongoing trials

The Harvard Women's Health Study (WHS) is a chemoprevention trial designed to evaluate the risks and benefits of low-dose aspirin, β-carotene and vitamin E in the primary prevention of cardiovascular disease and cancer in healthy postmenopausal women in the United States (Buring and Hennekens, 1993a,b). Begun in 1992, the WHS has enrolled approximately 40,000 female nurses, ages 45 and older, without a history of either disease. Participants are randomized to treatment or placebo groups for 2 years

following a 3-month non-randomized run-in phase. In response to the lack of benefit for β-carotene seen in completed trials, the WHS removed β-carotene supplementation from its intervention. The study will continue to evaluate aspirin and vitamin E.

Closed trials

This section summarizes five large-scale, randomized trials for which accrual has been either completed or closed and results reported; long-term follow-up is continuing for these trials to determine safety as well as efficacy (Omenn *et al*., 1996a).

The *Linxian Trials*, conducted by the NCI in collaboration with the Cancer Institute of the Chinese Academy of Medical Sciences, were two randomized, double-blind chemoprevention trials designed to determine whether daily ingestion of vitamin/mineral supplements would reduce incidence and mortality rates for oesophageal cancer in a high-risk population in Linxian, China, where approximately 20% of all deaths result from oesophageal cancer. The General Population Trial began in 1986 and randomized more than 29,000 individuals, who received one of four combinations of supplements, at doses equivalent to one to two times the US recommended daily allowances (RDAs), each day for 5 years. Combinations included retinal and zinc; riboflavin and niacin; vitamin C and molybdenum; and β-carotene, vitamin E and selenium. The second study, the Dysplasia Trial, enrolled 3318 individuals with evidence of severe oesophageal dysplasia; subjects were randomized to receive either a placebo or a daily supplement of 14 vitamins and 12 minerals, at two to three times the US RDAs, for 6 years.

Results of the General Population Trial indicated a significant benefit for those receiving the β-carotene/vitamin E/selenium combination – a 13% reduction in cancer mortality, due largely to a 21% drop in stomach cancer mortality (Blot *et al*., 1993). Also, this group experienced a 9% reduction in deaths from all causes, a 10% decrease in deaths from strokes, and a 4% decrease in deaths from oesophageal cancer. The effects of the β-carotene/vitamin E/selenium combination began to appear within 1–2 years after the intervention began and continued throughout the study; the three other combinations did not affect cancer risk.

A non-significant, 16% reduction in mortality from oesophageal cancer was reported for the Dysplasia Trial (Li *et al*., 1993). Analysis of oesophageal dysplasia data, however, showed that supplementation had a significant beneficial effect; individuals receiving supplements were 1.2 times more likely to have no dysplasia after 30 and 72 months of intervention, compared with individuals receiving the placebo (Mark *et al*., 1994). The results of these trials are encouraging but may not be directly applicable to Western cultures, which tend to be well nourished and not deficient in multiple micronutrients, in contrast with the Linxian community. Even so,

valuable information might be gained by determining cancer incidence for the 5 years since the trials ended, including analysis according to baseline nutrient levels (Omenn, 1998).

The *Physicians' Health Study* (*PHS*), a general population trial in 22,000 US physicians that evaluated the effect of aspirin and β-carotene supplementation on the primary prevention of cardiovascular disease and cancer, began in 1982. The dose of β-carotene was 50 mg on alternate days. The aspirin component of PHS ended in 1987, because a benefit of aspirin on risk of first heart attack (44% reduction) was found. The treatment period for β-carotene continued until December 1995; data showed no significant evidence of benefit or harm from β-carotene for either cardiovascular disease or cancer (Hennekens *et al*., 1996).

The *Alpha-Tocopherol, Beta-Carotene* (*ATBC*) *Cancer Prevention Study* and the *Beta-Carotene and Retinol Efficacy Trial* (*CARET*) both were carried out in populations at high risk for lung cancer. The ATBC Study investigated the efficacy of vitamin E (α-tocopherol, 50 mg) alone, β-carotene alone (20 mg), or a combination of the two compounds in preventing lung cancer among more than 29,000 male cigarette smokers ages 50–69, with an average treatment/followup of 6 years. Unexpectedly, this study showed a 16% higher incidence of lung cancer in the β-carotene group. However, 16% fewer cases of colorectal cancer were diagnosed among men who received vitamin E (ATBC Cancer Prevention Study Group, 1994; Albanes *et al*., 1996). Further, recent analysis of ATBC followup data found a 36% decrease in prostate cancer incidence and a 41% decrease in mortality from prostate cancer among men receiving vitamin E (Heinonen *et al*., 1998). In the ATBC Study, the adverse effects of β-carotene were observed at the highest two quartiles of ethanol intake, indicating that alcohol consumption may enhance the actions of β-carotene (Albanes *et al*., 1996). Also, follow-up data analysis showed a 32% lower incidence of prostate cancer among non-drinkers, whereas the risk among drinkers increased 25%, 42%, and 40% by tertiles (Heinonen *et al*., 1998). It is not clear how alcohol might influence the protective effect of β-carotene. CARET tested the efficacy of a combination of β-carotene (30 mg) and retinol (25,000 IU as retinyl palmitate) in former and current heavy smokers and in men with extensive occupational asbestos exposure. This trial was terminated in January 1996 after 4 years of treatment, when data showed an overall 28% higher incidence of lung cancer in participants receiving the β-carotene/retinyl palmitate combination (Omenn *et al*., 1996b). Male current smokers in CARET, excluding those exposed to asbestos, showed a 39% higher incidence (Omenn *et al*., 1996a), compared with the 16% higher incidence in the ATBC Study (Albanes *et al*., 1996), suggesting a possible adverse effect for supplemental retinol.

The results of these trials have led some to suggest that β-carotene should no longer be a candidate for inclusion in any future health interventions. Before such an extreme position is taken, however, a better understanding of the reasons for the unanticipated findings from ATBC and CARET is needed,

especially considering that no increase in lung cancer incidence was observed in the 11% of men in the PHS who were current smokers (Hennekens *et al*., 1996). Several possible explanations for the unanticipated outcomes of these β-carotene trials have been considered. The timing of the intervention may have been wrong (Erdmann *et al*., 1996; Omenn *et al*., 1996b; Rautalahti *et al*., 1997). The median follow-up of 6 years may have been too short, either to show any effect on carcinogenesis or to reverse or overcome lung cancer risk factors, particularly in active smokers. The continuing post-trial followup will help to clarify this issue (Omenn *et al*., 1996b; Rautalahti *et al*., 1997). Further, many heavy smokers and asbestos-exposed individuals may have developed the initial stages of lung cancer prior to supplementation. Although evidence prior to the trials suggested that β-carotene may be more effective at later stages of carcinogenesis, this might not be the case, and benefit might be minimal once initiation has occurred. Judging by the fact that the β-carotene effect appeared after only 2 years of supplementation, it is probable that the observed effect was related to the growth of cells that had already undergone malignant transformation (Erdmann *et al*., 1996; Rautalahti *et al*., 1997).

Inappropriate timing of the intervention, however, does not fully explain the excess risk observed in ATBC and CARET in individuals receiving supplements. Another consideration is that the high doses used (ATBC, 20 mg β-carotene; CARET, 30 mg β-carotene + 25,000 IU preformed vitamin A) may have had pro-oxidant effects rather than cancer-protective effects in combination with cigarette smoke and/or asbestos exposure (Erdmann *et al*., 1996; Mayne *et al*., 1996; Rautalahti *et al*., 1997). Direct oxidative attack on β-carotene by extremely reactive constituents of high-intensity cigarette smoke in the lungs of heavy smokers may induce the formation of β-carotene products that have pro-oxidant activity (Mayne *et al*., 1996). In asbestos workers, the inflammatory process in the asbestos-exposed lung – characterized by increased amounts of superoxide and hydrogen peroxide, both reactive oxygen species – may provide a favourable environment for the formation of pro-oxidant products of β-carotene (Mayne *et al*., 1996). Another possibility for the observed results is competitive inhibition by β-carotene of the antioxidant activity of other dietary carotenoids, such as α-carotene and lutein. α-Carotene, for example, has been reported to show higher potency than β-carotene in suppressing tumorigenesis in animal lung and skin models (Nishono, 1995).

It is noteworthy that participants with higher serum β-carotene concentrations at entry into the ATBC Study (Albanes *et al*., 1996) and CARET (Omenn *et al*., 1996a) developed fewer lung cancers during the course of the trials, even among those who received β-carotene supplements. Baseline serum concentrations of β-carotene reflect total intake of vegetables and fruits, which contain numerous other antioxidants, as well as many naturally occurring potential anticarcinogens that may exert their effects through diverse mechanisms; the β-carotene serum levels may simply be a marker for the actual protective agents. Thus, this finding is in agreement

with epidemiological evidence linking β-carotene-containing foods and lung cancer and re-affirms the importance of including an abundance of plant foods in our diets (Albanes *et al.*, 1996; Mayne *et al.*, 1996).

Future Directions

The cancer-protective effect of vegetables and fruits is not in question. Epidemiological evidence strongly and consistently supports such an effect. What is in question is which particular constituents in vegetables and fruits are responsible for the observed protective effect. Although it may be logical to believe that naturally occurring antioxidants in these plant foods play some role in reducing human cancer risk, it would be presumptuous to assume that antioxidants alone account for the cancer risk reduction. Is it possible to determine which constituents of foods are the effective agents? This question does not have a simple answer. It is likely that many constituents have important, but different roles with respect to carcinogenesis, depending on cancer site, cancer stage and prevailing risk factors (Block, 1993). Also, doses of substances delivered to any specific tissue, and hence their effects, will be determined in part by genetic metabolic profiles that vary across populations and within individuals (Potter, 1996). Considering the variety of phyto-chemicals in commonly consumed plant foods and the possibility that they may act simultaneously, the interactive effects of these substances could be significant, as well as complex and difficult to unravel.

Although sorting out the cancer-related effects of vegetable and fruit constituents and the mechanisms by which they exert these effects may seem overwhelming, the potential benefits of such research endeavours for human health are great and will be well worth the multidisciplinary approaches and collaborative efforts required. Large gaps exist in our basic knowledge of even the most widely studied substances. Carotenoids, including β-carotene, illustrate this point well. As noted by the Carotenoid Research Interactive Group (CARIG) (Erdmann *et al.*, 1996), an international organization of scientists, basic information is lacking about digestion and absorption of carotenoids from foods, carotenoid bioavailability and metabolic fate, the role of *cis*-carotene isomers as provitamin A, effects of carotenoids on cellular metabolism and immunity, gene/carotenoid interactions, *in vivo* antioxidant value of carotenoids, composition of carotenoids in foods, and effects of simple food preparation techniques on carotenoid chemistry. Also, inadequate clinical research data are available about which types of cancer might be responsive to carotenoids, optimal carotenoid mixtures and dose levels, timing of dose in relationship to carcinogenic stages, characteristics of individuals who might be responsive, and appropriate pharmacokinetic and safety data (Erdmann *et al.*, 1996). Comprehensive, yet well-targeted, clinical trials and other research approaches that aim to answer such questions for nutritive and non-nutritive

constituents of plant foods, including the carotenoids and other antioxidants, are essential to accelerate progress in cancer prevention research and to achieve definitive results that can be translated into effective, practical cancer-preventive applications for the general population.

References

Ahmad, N., Feyes, D.K., Niemenen, A.-L., Agarwal, R. and Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *Journal of the National Cancer Institute* 89, 1881–1886.

Albanes, D., Heinonen, O.P., Taylor, P.R., Virtamo, J., Edwards, B.K., Rautalahti, M, Hartman, A.M., Palmgren, J., Freedman, L.S., Haapakoski, J., Barrett, M.J., Pietinen, P., Malila, N., Tala, E., Liippo, K., Salomaa, E.-R., Tangrea, J.A., Teppo, L., Askin, F.B., Taskinen, E., Erozan, Y., Greenwald, P. and Huttunen, J.K. (1996) α-Tocopherol and β-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: effects of base-line characteristics and study compliance. *Journal of the National Cancer Institute* 88, 1560–1570.

Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine* 330, 1029–1035.

Block, G. (1991) Vitamin C and cancer prevention: the epidemiologic evidence. *American Journal of Clinical Nutrition* 53, 270S–282S.

Block, G. (1993) Micronutrients and cancer: time for action? (editorial). *Journal of the National Cancer Institute* 85, 846–848.

Block, G., Patterson, B.H. and Subar, A.F. (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer* 18, 1–29.

Blot, W.J., Li, J.-Y., Taylor, P.R., Guo, W., Dawsey, S.M., Wang, G.-Q., Yang, C.S., Zheng, S.-F., Gail, M.H., Li, G.-Y., Yu, Y., Liu, B.-Q., Tangrea, J.A., Sun, Y.-H., Liu, F., Fraumeni, J.F. Jr, Zhang, Y.-H. and Li. B. (1993) Nutrition intervention trials in Linxian: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *Journal of the National Cancer Institute* 85, 1483–1492.

Buring, J.E. and Hennekens, C.H. (1993a) The Women's Health Study: summary of the study design. *Journal of Myocardial Ischemia* 4, 27–29.

Buring, J.E. and Hennekens, C.H. (1993b) The Women's Health Study: rationale and background. *Journal of Myocardial Ischemia* 4, 30–40.

Byers, T. and Guerrero, N. (1995) Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *American Journal of Clinical Nutrition* 62, 1385S–1392S.

Chug-Ahuja, J.K., Holden, J.M., Forman, M.R., Mangels, A.R., Beecher, G.R. and Lanza, E. (1993) The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. *Journal of the American Dietetic Association* 93, 318–323.

Clark, L.C., Combs, G.F. Jr, Turnbull, B.W., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Glover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Lesher, J.L. Jr, Park,

H.K., Sanders, B.B. Jr, Smith, C.L. and Taylor, J.R. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *Journal of the American Medical Association* 276, 1957–1963.

Clinton, S.K., Emenhiser, C., Schwartz, S.J., Bostwick, D.G., Williams, A.W., Moore, B.J. and Erdman, J.W. Jr (1996) *cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiology, Biomarkers and Prevention* 5, 823–833.

Cozzi, R., Ricordy, R., Aglitti, T., Gatta, V., Perticone, P. and De Salvia, R. (1997) Ascorbic acid and β-carotene as modulators of oxidative damage. *Carcinogenesis* 18, 223–228.

Diplock, A.T. (1996) Antioxidants and disease prevention. *Food and Chemical Toxicology* 34, 1013–1023.

Erdman, J.W. Jr, Russell, R.M., Mayer, J., Rock, C.L., Barua, A.B., Bowen, P.E., Burri, B.J., Curran-Celentano, J., Furr, H., Mayne, S.T. and Stacewicz-Sapuntzakis, M. (1996) Beta-carotene and the carotenoids: beyond the intervention trials. *Nutrition Reviews* 54, 185–188.

Garewal, H.S., Meyskens, F.L. Jr, Killen, D., Reeves, D., Kiersch, T.A., Elletson, H., Strosberg, A., King, D. and Steinbronn, K. (1990) Response of oral leukoplakia to beta-carotene. *Journal of Clinical Oncology* 8, 1715–1720.

Giovannucci, E., Ascherio, A., Rimm, E.B., Stampfer, M.J., Colditz, G.A. and Willett, W.C. (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. *Journal of the National Cancer Institute* 87, 1767–1776.

Giuliano, A.R. and Gapstur, S. (1998) Can cervical dysplasia and cancer be prevented with nutrients? *Nutrition Reviews* 56, 9–16.

Greenberg, E.R., Baron, J.A., Tosteson, T.D., Freeman, D.H. Jr, Beck, G.J., Bond, J.H., Colacchio, T.A., Coller, J.A., Frankl, H.D., Haile, R.W., Mandel, J.S., Nierenberg, D.W., Rothstein, R., Snover, D.C., Stevens, M.M., Summers, R.W. and van Stolk, R.U. (1994) A clinical trial of antioxidant vitamins to prevent colorectal adenoma. *New England Journal of Medicine* 331, 141–147.

Heinonen, O.P., Albanes, D., Virtamo, J., Taylor, P.R., Huttenen, J.K., Hartman, A.M., Haapakoski, J., Malila, N., Rautalahti, M., Ripatti, S., Mäenpää, H., Teerenhovi, L., Koss, L., Virolainen, M. and Edwards, B.K. (1998) Prostate cancer and supplementation with α-tocopherol and β-carotene: incidence and mortality in a controlled trial. *Journal of the National Cancer Institute* 90, 440–446.

Helzlsouer, K.J., Alberg, A.J., Norkus, E.P., Morris, J.S., Hoffman, S.C. and Comstock, G.W. (1996) Prospective study of serum micronutrients and ovarian cancer. *Journal of the National Cancer Institute* 88, 32–37.

Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., LaMotte, F., Gaziano, J.M., Ridker, P.M., Willett, W. and Peto, R. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine* 334, 1145–1149.

Hunter, D.J. and Willett, W.C. (1996) Nutrition and breast cancer. *Cancer Causes and Control* 7, 56–68.

Imai, K., Suga, K. and Nakachi, K. (1997) Cancer-preventive effects of drinking green tea among a Japanese population. *Preventive Medicine* 26, 769–775.

Jacob, R.A. and Burri, B.J. (1996) Oxidative damage and defense. *American Journal of Clinical Nutrition* 63, 985S–990S.

Ji, B.-T., Chow, W.-H., Hsing, A.W., McLaughlin, J.K., Dai, Q., Gao, Y.-T., Blot, W.J. and Fraumeni, J.F. Jr. (1997) Green tea consumption and the risk of pancreatic and colorectal cancers. *International Journal of Cancer* 70, 255–258.

Kelloff, G.J. and Boone, C.W. (eds) (1994) Clinical development plan: β-carotene and other carotenoids. *Journal of Cellular Biochemistry* 20S, 110–140.

Kelloff, G.J. and Boone, C.W. (eds) (1996a) Clinical development plan: 1,4-phenylenebis-(methylene) selenocyanate. *Journal of Cellular Biochemistry* 26S, 219–226.

Kelloff, G.J. and Boone, C.W. (eds) (1996b) Clinical development plan: curcumin. *Journal of Cellular Biochemistry* 26S, 72–85.

Kelloff, G.J., Boone, C.W., Crowell, J.A., Steele, V.E., Lubert, R. and Sigman, C.C. (1994) Chemopreventive drug development: perspectives and progress. *Cancer Epidemiology, Biomarkers and Prevention* 3, 85–98.

Khachik, F., Beecher, G.R. and Smith, J.C. Jr (1995) Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *Journal of Cellular Biochemistry* 22S, 236–246.

Kimmick, G.G., Bell, R.A. and Bostick, R.M. (1997) Vitamin E and breast cancer: a review. *Nutrition and Cancer* 27, 109–117.

Kolonel, L.N. (1996) Nutrition and prostate cancer. *Cancer Causes and Control* 7, 83–94.

Kono, S. and Hirohata, T. (1996) Nutrition and stomach cancer. *Cancer Causes and Control* 7, 41–55.

La Vecchia, C., Ferraroni, M., Negri, E. and Franceschi, S. (1998) Role of various carotenoids in the risk of breast cancer. *International Journal of Cancer* 75, 482–483.

Le Marchand, L., Hankin, J.H., Bach, F., Kolonel, L.N., Wilkens, L.R., Stacewicz-Sapuntzakis, M., Bowen, P.E., Beecher, G.R., Laudon, F., Baque, P., Daniel, R., Seruvatu, L. and Henderson, B.E. (1995) An ecological study of diet and lung cancer in the South Pacific. *International Journal of Cancer* 63, 18–23.

Li, J.-Y., Taylor, P.R., Dawsey, S.M., Wang, G.-Q., Ershow, A.G., Guo, W., Liu, S.-F., Yang, C.S., Shen, Q., Wang, W., Mark, S.D., Zou, X.-N., Greenwald, P., Wu, Y.-P. and Blot, W.J. (1993) Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *Journal of the National Cancer Institute* 85, 1492–1498.

Lippman, S.M., Batsakis, J.G., Toth, B.B., Weber, R.S., Lee, J.J., Martin, J.W., Hays, G.L., Goepfert, H. and Hong, W.K. (1993) Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. *New England Journal of Medicine* 328, 15–20.

Loft, S. and Poulsen, H.E. (1996) Cancer risk and oxidative DNA damage in man. *Journal of Molecular Medicine* 74, 297–312.

Lubin, F., Rozen, P., Arieli, B., Farbstein, M., Knaani, Y., Bat, L. and Farbstein, H. (1997) Nutritional and lifestyle habits and water–fiber interaction in colorectal adenoma etiology. *Cancer Epidemiology, Biomarkers and Prevention* 6, 79–85.

MacClennan, R., Macrae, F., Bain, C., Battistutta, D., Chapuis, P., Gratten, H., Lambert, J., Newland, R.C., Ngu, M., Russell, A., Ward, M. and Wahlqvist, M.L. (1995) Randomized trial of intake of fat, fiber, and beta carotene to prevent colorectal adenomas. *Journal of the National Cancer Institute* 87, 1760–1766.

Manna, C., Galletti, P., Cucciolla, V., Moltedo, O., Leone, A. and Zappia, V. (1997) The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)-ethanol

counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *Journal of Nutrition* 127, 286–292.

Mark, S.D., Liu, S.-F., Li, J.-Y., Gail, M.H., Shen, Q., Dawsey, S.M., Liu, F., Taylor, P.R., Li, B. and Blot, W.J. (1994) The effect of vitamin and mineral supplementation on esophageal cytology: results from the Linxian Dysplasia Trial. *International Journal of Cancer* 57, 162–166.

Mayne, S.T., Handelman, G.J. and Beecher, G. (1996) β-carotene and lung cancer promotion in heavy smokers – a plausible relationship? *Journal of the National Cancer Institute* 88, 1513–1515.

Nishono, H. (1995) Cancer chemoprevention by natural carotenoids and their related compounds. *Journal of Cellular Biochemistry* 22S, 231–235.

Nomura, A.M.Y., Ziegler, R.G., Stemmermann, G.N., Chyou, P.-H. and Craft, N.E. (1997a) Serum micronutrients and upper aerodigestive tract cancer. *Cancer Epidemiology, Biomarkers and Prevention* 6, 407–412.

Nomura, A.M.Y., Stemmermann, G.N., Lee, J. and Craft, N.E. (1997b) Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. *Cancer Epidemiology, Biomarkers and Prevention* 6, 487–491.

Omenn, G.S. (1998) Interpretation of the Linxian vitamin supplement chemoprevention trials. *Epidemiology* 9, 1–3.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L. Jr, Valanis, B., Williams, J.H. Jr, Barnhart, S., Cherniack, M.G., Brodkin, C.A. and Hammar, S. (1996a) Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *Journal of the National Cancer Institute* 88, 1550–1559.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L. Jr, Valanis, B., Williams, J.H. Jr, Barnhart, S. and Hammar, S. (1996b) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine* 334, 1150–1155.

Perera, F.P. (1996) Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. *Journal of the National Cancer Institute* 88, 496–509.

Peto, R., Doll, R., Buckley, J.D. and Sporn, M.B. (1981) Can dietary beta-carotene materially reduce human cancer rates? *Nature* 290, 201–208.

van Poppel, G. and Goldbohm, R.A. (1995) Epidemiologic evidence for β-carotene and cancer prevention. *American Journal of Clinical Nutrition* 62, 1393S–1402S.

Potter, J.D. (1996) Food and phytochemicals, magic bullets and measurement error: a commentary. *American Journal of Epidemiology* 144, 1026–1027.

Potischman, N. and Brinton, L.A. (1996) Nutrition and cervical neoplasia. *Cancer Causes and Control* 7, 113–126.

Rautalahti, M., Albanes, D., Virtamo, J., Taylor, P.R., Huttunen, J.K. and Heinonen, O.P. (1997) Beta-carotene did not work: aftermath of the ATBC Study. *Cancer Letters* 114, 235–236.

Reddy, B.S., Rivenson, A., El-Bayoumy, K., Upadhyaya, P., Pittman, B. and Rao, C.V. (1997) Chemoprevention of colon cancer by organoselenium compounds and impact of high- or low-fat diets. *Journal of the National Cancer Institute* 89, 506–512.

Schrauzer, G.N., White, D.A. and Schneider, C.J. (1977) Cancer mortality correlation

studies. III. Statistical associations with dietary selenium intakes. *Bioinorganic Chemistry* 7, 23–34.

Steele, V.E., Sharma, S., Mehta, R., Elmore, E., Redpath, L., Rudd, C., Bagheri, D., Sigman, C.C. and Kelloff, G.J. (1996) Use of *in vitro* assays to predict the efficacy of chemopreventive agents in whole animals. *Journal of Cellular Biochemistry* 26S, 29–53.

Steinmetz, K.A. and Potter, J.D. (1996) Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association* 96, 1027–1039.

Stich, H.F., Stich, W., Rosin, M.P. and Vallejera, M.O. (1984) Use of the micronucleus test to monitor the effect of vitamin A, beta-carotene and canthaxanthin on the buccal mucosa of betel nut/tobacco chewers. *International Journal of Cancer* 34, 745–750.

Stone, W.L. and Papas, A.M. (1997) Tocopherols and the etiology of colon cancer. *Journal of the National Cancer Institute* 89, 1006–1014.

Stoner, G.D. and Mukhtar, H. (1995) Polyphenols as cancer chemopreventive agents. *Journal of Cellular Biochemistry* 22S, 169–180.

Tseng, M., Murray, S.C., Kupper, L.L. and Sandler, R.S. (1996) Micronutrients and the risk of colorectal adenomas. *American Journal of Epidemiology* 144, 1005–1014.

Visioli, F., Bellomo, G., Montedoro, G. and Galli, C. (1995) Low density lipoprotein oxidation is inhibited *in vitro* by olive oil constituents. *Atherosclerosis* 117, 25–32.

Wattenberg, L.W. (1992) Inhibition of carcinogenesis by minor dietary constituents. *Cancer Research* 52, 2085S–2091S.

Yang, C.S., Chen, L., Lee, M.-J. and Landau, J.M. (1996) Effects of tea on carcinogenesis in animal models and humans. In: American Institute for Cancer Research (ed.) *Dietary Phytochemicals in Cancer Prevention and Treatment*. Plenum Press, New York, pp. 51–61.

Ziegler, R.G., Mayne, S.T. and Swanson, C.A. (1996a) Nutrition and lung cancer. *Cancer Causes and Control* 7, 157–177.

Ziegler, R.G., Colavito, E.A., Hartge, P., McAdams, M.J., Schoenberg, J.B., Mason, T.J. and Fraumeni, J.F. Jr. (1996b) Importance of α-carotene, β-carotene, and other phytochemicals in the etiology of lung cancer. *Journal of the National Cancer Institute* 88, 612–615.