6. Carcinogenicity

6.1 Humans

Two of the intervention trials described in section 4.1.1.3 suggest increased incidences of lung cancer. These are discussed below. Non-significant increases in the incidences of cancers at other sites in intervention trials and case–control studies are mentioned in the full descriptions of these studies in section 4.1.1.3.

6.1.1 ATBC Study

In the ATBC study (Alpha-Tocopherol, Beta Carotene Cancer Prevention Group, 1994a,b; Albanes *et al.*, 1996), involving 29 133 men who smoked at least five cigarettes daily at entry, with a median of 20 cigarettes per day and a median duration of smoking of 36 years, 894 cases of lung cancer were identified during the follow-up. The incidence of lung cancer was 16% higher among the men who received β-carotene than among those who did not (RR, 1.16; 95% CI, 1.02–1.33). The effect appeared to be stronger, but not significantly so, in participants who smoked at least 20 cigarettes daily at entry (RR, 1.25; 95% CI, 1.07–1.46) than in those who smoked 5–19 cigarettes daily (RR, 0.97; 95% CI, 0.76–1.23), and in those with higher alcohol intake (≥ 11 g of ethanol daily) (RR, 1.35; 95% CI, 1.01–1.81) than in those with a lower intake (RR, 1.03; 95% CI, 0.85–1.24). A 23% increase in the incidence of prostate cancer and a 25% increase of stomach cancer were also seen among men receiving β-carotene in comparison with those not receiving β-carotene, but these differences were not statistically significant; the incidences of cancers of the colon and rectum and urinary bladder were not affected by β-carotene.

6.1.2 CARET

The CARET (Omenn *et al.*, 1996a,b) involved 14 254 men and women 50–69 years of age who had at least 20 pack–years of cigarette smoking and either were currently smoking or had stopped smoking within the previous six years; 4060 men with substantial occupational exposure to asbestos were also included. Daily supplementation with β-carotene and retinyl palmitate was stopped 21 months early, after a

median of 3.7 years of follow-up, because of clear evidence of no benefit and substantial evidence of possible harm. A total of 388 new cases of lung cancer were diagnosed during the follow-up. The actively treated group had a relative risk for lung cancer of 1.28 (95% CI, 1.04–1.57) as compared with those on placebo. This overall risk for lung cancer included relative risks of 1.40 (95% CI, 0.95–2.07) for workers exposed to asbestos, 1.42 (95% CI, 1.07–1.87) for heavy smokers who were smoking at the time of randomization and 0.80 (95% CI, 0.48–1.31) for previously heavy smokers who were no longer smoking at the time of randomization. In comparison with the group given placebo, those receiving the supplement in the highest quartile of alcohol intake had an increased risk for lung cancer (RR, 1.99; 95% CI, 1.28–3.09); a test for the heterogeneity of relative risks among quartiles of alcohol intake was statistically significant at the 0.01 level. Because of the lack of a consistent dose– response effect and the multiple tests performed, the authors considered this result to be only suggestive. There were no statistically significant differences in the risks for cancers at other sites.

6.1.3 Interpretation of trials suggesting carcinogenicity

The groups receiving β-carotene supplementation had an increased risk for lung cancer in both the ATBC Study and CARET (in which the group also received retinyl palmitate). No change in lung cancer risk was found in the Linxian or Physicians' Health studies, but their power to find a small change in lung cancer risk was limited by the relatively small number of cases of lung cancer. There was no statistically significant increase in the incidence of any other cancer in these studies. Thus, the question pertains to the possibility that β-carotene supplementation increased the risk for developing lung cancer.

Differences in the study populations and interventions may explain some of the results of the trials. In the ATBC Study and the PHS, all of the participants were men, whereas in the CARET and Linxian studies, half of the participants were women. Supplementation in the PHS was with β-carotene alone at 50 mg every second day; in the ATBC study, β-carotene was given at 20 mg per day either alone or in combination with α-tocopherol; in the CARET, β-carotene was given at 30 mg per day in combination with vitamin A; and in Linxian, a combination of β-carotene at 15 mg per day, vitamin E and selenium was given. In the ATBC study, all of the participants were smokers, in the CARET, 60%, in the Linxian study, 30% and in PHS, only 11%. The CARET suggested increased risks for lung cancer among current smokers and asbestos-exposed workers but not among former smokers. In the ATBC study, the increased risk for lung cancer appeared to be confined to people smoking at least 20 cigarettes per day, and no effect on risk was found for those smoking 5–19 cigarettes per day (although the test for effect modification was not statistically significant). Years of cigarette smoking did not significantly modify the effect of β-carotene supplementation on lung cancer risk. These findings indicate that β-carotene supplementation accelerates the clinical appearance of lung cancer among current, heavy smokers. The ATBC study and CARET suggest that alcohol intake may modify the effect of β-carotene on lung cancer. The intake levels were, however, moderate: the median in the ATBC study was 11 g/day and that in the CARET, 3 g/day. In the ATBC study, the effect of β-carotene appeared to be stronger in men with a higher alcohol intake than in those with a lower intake; however, underestimation of alcohol intake is likely the higher the intake is. The CARET also suggested that the risk for lung cancer was greater in people with a high alcohol intake, but there was no consistent dose–response relationship. The separate effects of alcohol drinking and tobacco smoking are difficult to distinguish, as the two behaviours are correlated.

Other potentially important differences between the completed trials include baseline nutrient status and the population's response to the β-carotene intervention. As shown in Table 56, the population in the Linxian County study had notably low β-carotene concentrations at entry; in contrast, the physicians enrolled in the PHS had notably high β-carotene concentrations at entry. The study populations also varied in their plasma

Table 56. Serum concentrations of β-carotene before and after intervention in completed cancer prevention trials

Study	Serum β-carotene at entry (μg/dl)	Serum β-Carotene after intervention (μg/dl)	β-Carotene dose (manufacturer)
ATBC	[17][a]	[300][a]	20 mg/d (Roche)
CARET	[17][a,b]	[210][a]	30 mg/d (Roche)
PHS	[30][b,c]	[120][c]	50 mg on alternate days (BASF)
Linxian	6[c]	86[c]	15 mg/d (Roche)

ATBC, Alpha-Tocopherol Beta-Carotene; CARET, Beta-Carotene Retinol Efficacy Trial; PHS, Physicians' Health Study

[a] Median

[b] Level in placebo group after intervention

[c] Mean

responses to supplemental β-carotene. The participants in the two trials that provide evidence of potential harm (CARET and ATBC) also had the highest serum concentrations of β-carotene at the end of the intervention and those in the PHS and Linxian studies notably lower concentrations. The dose of β-carotene and the median serum β-carotene concentrations achieved in these studies exceeded by many times the dietary intake or serum concentration of β-carotene associated with a lowered risk for cancer in the epidemiological follow-up studies.

6.2 Experimental animals

Mouse: It was reported in an abridged paper that beadlets containing 11.5% β-carotene were incorporated into the diet of four groups of 100 male and 100 female CD-1 mice [age unspecified] at concentrations resulting in β-carotene doses of 100, 250, 500 or 1000 mg/kg bw per day [formulation in the diet unspecified]. Two groups of mice were fed either unsupplemented standard diet or standard diet supplemented with placebo beadlets. Administration of β-carotene for up to 105 weeks did not affect the spontaneous tumour profile [no further information was given on mortality or tumours] (Heywood *et al.*, 1985).

Rat: Two groups of 15 male and 15 female Wistar rats, 30–40 days old, were fed a 'synthetic' diet for 90 weeks followed by ground commercial laboratory chow pellets for another 20 weeks, either as such or supplemented with 0.1% β-carotene (96% all-*trans* isomer) [formulation in the diet unspecified]. After one year, four rats [sex unspecified] of each group were killed for interim observations. There was no significant difference in body weight between the two groups. At termination of the study at week 110, 7/26 controls [sex unspecified] and 13/26 rats fed β-carotene-fed [sex unspecified] were still alive. Extensive histopathological examination of the survivors revealed no treatment-related changes, except for storage of Sudan-positive material in the Kupffer cells of the livers of the rats fed β-carotene (Zbinden & Studer, 1958; Bagdon *et al.*, 1960) [The Working Group noted the small number of animals per group, the short treatment period, the absence of histopathological examination of the animals that died intercurrently and the lack of information on spontaneous tumours.]

It was reported in an abridged paper that beadlets containing about 11.5% β-carotene were incorporated into the diet of Sprague-Dawley rats at concentrations resulting in doses of 100, 250, 500 and 1000 mg/kg bw per day for life; other rats received either unsupplemented standard diet or standard diet supplemented with placebo beadlets. The F_{1a} pups

were assigned to six groups of 60 males and 60 females, each receiving the same diet as their mothers. After 78 weeks, when the clotting times in all male rats appeared to be prolonged, 15 ppm heterazeen (a biologically active vitamin K analogue) was added to the diet of all rats. All survivors were killed when 20% of the controls on standard diet were still alive (week 116 for males and week 114 for females). The body-weight gain of animals at the three highest doses was lower than that of rats receiving placebo [no further details given; statistics unspecified]. β-Carotene did not affect the spontaneous tumour profile [no further information on mortality or tumours] (Heywood *et al.*, 1985).

No data were available to the Working Group on other carotenoids.

6.3 Mechanisms of carcinogenicity

Mechanisms to explain the excess incidence of lung cancers observed in persons receiving β-carotene supplements in the CARET and the ATBC study remain speculative. Possible mechanisms of effect to account for the observed excess, assuming that it is in fact real and attributable to β-carotene, are discussed below.

The results of the two trials strongly suggest that concurrent exposure to β-carotene and to a relatively high intensity of cigarette smoke is necessary for a harmful effect of β-carotene to occur. Moreover, the risk appears to be specific to lung cancer. The combination of tobacco smoke, which contains many free radicals and is strongly oxidative, and relatively high partial pressures of oxygen in the lung may trigger autooxidation of β-carotene and other carotenoids in the lung (discussed by Mayne *et al.*, 1996; see also Section 2). Under such conditions, the free radical of β-carotene may also serve as a propagator of free-radical formation. Transformed or damaged cells found in the lungs of long-term smokers might be particularly sensitive to either modulation of the oxidative state or the non-physiological concentrations of β-carotene present. This hypothesis is consistent with the work of Leo *et al.* (1992), which showed that ethanol-induced hepatotoxicity in a baboon model was exacerbated by a challenge of non-physiological doses of β-carotene. In this situation as well, damaged cells seem more susceptible to the adverse effects of β-carotene.

The amount of β-carotene that is autooxidized is known to be dose-dependent (see Table 56 for blood concentrations in this and other trials), and that might explain the lack of effect on lung cancer rates in the PHS. Similarly, as discussed elsewhere (Mayne *et al.*, 1996), asbestos fibres also produce an inflammatory response in lung, resulting in overproduction of reactive oxygen species.

The link between local oxidative stress in the lung and promoting effects of supplemental β-carotene is unclear; however, several critical regulatory pathways and signalling molecules are redox-regulated (e.g. NF-kB, and AP-1). Thus, it can be hypothesized that a pro-oxidant state in the lung, due to autooxidation of β-carotene and/or severe oxidative stress, might result in alterations in both cell proliferation and apoptosis. Liebler (1993), however, used in-vitro models of β-carotene and cigarette smoke to show that autooxidation of β-carotene occurs after exposure to tobacco but does not result in a prooxidant state except in models with β–carotene (Omaye *et al.*, 1997). This might suggest that oxidative metabolites of β-carotene, rather than a prooxidative state, are responsible for any effects observed.

Further considerations are the following:

- High concentrations of β-carotene present in the gastrointestinal tract at the same time as other carotenoids may inhibit the absorption of other protective phytochemicals, such as α-carotene and lutein. Although competitive interactions have been shown between various carotenoids when administered together in large amounts (Kostic *et al.*, 1995), large recurrent doses of β-carotene had little effect on other serum carotenoids in intervention trials (Albanes *et al.*, 1997; Mayne *et al.*, 1997).
- Cigarette smoke may activate leukocytes and macrophages to secrete oxidizing agents, with formation of carotenyl radical adducts by O_2^- (Trush & Kensler, 1991).

- β-Carotene may inhibit the apoptosis of preneoplastic or neoplastic cells, thus enhancing the survival of such cells. Supplements of β-carotene decreased apoptosis (Johnson *et al.*, 1996; Mannick *et al.*, 1996).
- β-Carotene may be an effective antioxidant in lung, thereby altering the redox state and potentially affecting redox-regulated aspects of cell proliferation and apoptosis. The lack of an effect (e.g. lung carcinogenicity) of vitamin E in the ATBC trial suggests, however, that this possibility is not likely.

These mechanisms are not mutually exclusive, and more than one may be involved.