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Serum beta-carotene and subsequent risk of cancer: Results from the BUPA Study

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Summary In the BUPA Study, a prospective study of 22,000 men attending a screening centre in London, serum samples were collected and stored. The concentration of beta-carotene was measured in the stored serum samples from 271 men who were subsequently notified as having cancer and from 533 unaffected controls, matched for age, smoking history and duration of storage of the serum samples. The mean beta-carotene level of the cancer subjects was significantly lower than that of their matched controls (198 and 221 $\mu g l^{-1}$ respectively, $P=0.007$). The difference was apparent in subjects from whom blood was collected several years before the diagnosis of the cancer, indicating that the low beta-carotene levels in the cancer subjects were unlikely to have been simply a consequence of pre-clinical disease. Men in the top two quintiles of serum beta-carotene had only about 60% of the risk of developing cancer compared with men in the bottom quintile. The study was not large enough to be able to indicate with confidence the sites of cancer for which the inverse association between serum beta-carotene and risk of cancer applied, though the association was strongest for lung cancer. The association may be due to beta-carotene affecting the risk directly or it may reflect an indirect association of cancer risk with some other component of vegetables or with a non-vegetable component of diet that is itself related to vegetable consumption.

It has been suggested that beta-carotene (and other carotenoids) may play a role in reducing the incidence of cancer (Peto *et al.*, 1981). Beta-carotene has anti-oxidant activity (Burton & Ingold, 1984); it is an efficient quencher of singlet oxygen (Foote, 1979). Singlet oxygen is a toxic, and possibly cancer inducing, form of oxygen that occurs as a result of many metabolic reactions.

There is limited evidence to suggest that beta-carotene supplementation reduces the risks of chemically induced tumours in animals. Since an important function of carotenoid pigments is to protect organisms from photosensitisation, and hence probably skin cancer which can occur as a result, the administration of beta-carotene to animals is likely to reduce the incidence of skin cancers occurring in those animals. What is, perhaps, of greater interest is whether the administration of beta-carotene can reduce the incidence of cancers that are not induced by ultraviolet light, and in particular of cancers that affect tissues other than skin. Dorogokupla (cited by Mathews-Roth, 1982) induced subcutaneous tumours in rats with injections of 9,10-dimethyl-1-2-benzanthracene (DMBA) and skin tumours in mice by the topical application of DMBA; animals fed a diet supplemented with unlimited amounts of red carrots developed tumours at a lower rate than did the animals receiving the unsupplemented diet. Mathews-Roth (1982) administered about 6.7 grams of beta-carotene per kilogram of diet per day to mice and showed that this led to a considerable increase in pigment accumulation in the skin (593 mg $100\,g^{-1}$ skin) and that the induction of skin tumours by 7,12-dimethylbenzanthracene promoted by croton oil, was inhibited by the stored beta-carotene, there being 3.4 tumours per mouse in the beta-carotene group compared to 11.6 tumours per mouse in the control group ($P<0.01$). Mathews-Roth used canthaxanthin, a carotenoid without vitamin A activity, as a control substance. It showed no significant anti-cancer activity. Rettura and his colleagues (1982) found that beta-carotene (and retinyl palmitate) reduced the growth of implanted adenocarcinoma cells. Temple & Basu (1987) demonstrated significantly less colon cancers, in 1,2-dimethylhydrazine treated mice given high dose ($22\,mg\,kg^{-1}$) beta-carotene compared with similarly treated mice given a lower dose ($2\,mg\,kg^{-1}$ body weight).

The dietary epidemiological studies have consistently shown that people with a relatively low intake of beta-carotene (or total carotenoids) have a high risk of cancer, notably lung cancer. This was found to be the case in almost every study, most of which were retrospective but some prospective (Stocks, 1958; Bjelke, 1975; Phillips, 1975; MacLennan *et al*, 1977; Bjelke, 1978; Tuyns *et al.*, 1978; Cook-Mozaffari *et al.*, 1979; Hirayama, 1979; Mettlin *et al.*, 1979; Gregor *et al.*, 1980; Mettlin *et al.*, 1981; Modan *et al.*, 1981; Shekelle *et al.*, 1981; Kvale *et al.*, 1983; Byers *et al.*, 1984; Hinds *et al.*, 1984; Ziegler *et al.*, 1984; Long-De W. *et al.*, 1985; Samet *et al.*, 1985; Stehr *et al.*, 1985; Wu *et al.*, 1985; Pisani *et al.*, 1986; Kolonel *et al.*, 1987). For lung cancer, the results are clear-cut; the relative risk lying between 1.5 and 2.5 among those with low estimated beta-carotene intakes compared with those with a high intake. For cancer of the stomach, large bowel and oesophagus, the relative risk was about 1.5. For cancer of the larynx, bladder and prostate, the relative risk lay between 2 and 3. Most of the studies have been considered in an earlier review (Wald, 1982); more recent studies have been generally confirmatory.

Unlike dietary retinol, which is not an important determinant of serum retinol levels in well nourished populations (Willet *et al.*,1984*a*; Wald *et al.*, 1985), dietary beta-carotene has a strong influence on serum beta-carotene levels (Willet *et al.*, 1983). Since dietary beta-carotene is inversely associated with the risk of cancer, and dietary beta-carotene is associated with serum beta-carotene levels, it follows that one would expect to find an inverse association between serum beta-carotene and the risk of cancer. To see if this was indeed the case, we conducted a prospective study of serum beta-carotene levels in men attending a medical screening centre in London.

Subjects and methods

The design of the prospective study has been described before (Wald *et al.*, 1980, 1986). In summary, blood was collected from about 22,000 men aged 35–64 years who,

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between 1975 and 1982, attended the British United Provident Association (BUPA) Medical Centre in London for a comprehensive medical examination. Serum was separated from the blood sample and stored at −40°C. The National Health Service records of these men were flagged and, through the assistance of the Office of Population Censuses and Surveys, notification was received in the event of a diagnosis of cancer or death. By April 1985, 271 men who had provided sufficient serum for beta-carotene analysis were identified as having developed cancer (subjects). Two controls were selected for each of the subjects, matched on age (within 5 years), duration of storage of the serum sample (within 3 months), smoking habits (current smoker, ex-smoker or life-long non-smoker) and, for current smokers, smoking habits – type of product smoked (cigarette, cigar or pipe), amount smoked (within 5 cigarettes per day, 2 cigars per day or an ounce of tobacco per week) and age of starting to smoke (within 5 years). In this way samples from 533 matched controls were identified and analysed. This was 9 less than the 'expected' 542 because serum from some of the cases and controls was spoilt in transport prior to assay; if a case or both controls were so affected all three were omitted but if only one control was so affected the remaining matched case and control were retained in the analysis. The beta-carotene estimations were performed by high pressure liquid chromatography (Vuilleumier *et al.*, 1983).

Samples were tested in four separate series, two in 1981, one in 1983 and one in 1985. Sera from subjects and their matched controls were always assayed in the same analytical batch without knowledge of whether they were from subjects or from controls. The statistical analysis was based on logarithmically transformed values of beta-carotene, their overall distribution being approximately Gaussian after transformation. All the mean values of beta-carotene presented are transformed back from the logarithmic scale and the standard errors given are approximate. The mean values are adjusted for series, to take account of any changes in assay performance between series, but the (2-sided) p-values given for comparing these means are derived from analyses of variance adjusting for all the variables on which the matching of cases and controls was based. Relative risks were estimated by logistic regression, using the method of Breslow and Day (1980) which takes into account the factors included in the matching.

Results

Table I shows the mean serum beta-carotene concentration of subjects and matched controls both overall and classified according to the site of the cancer. The mean beta-carotene concentration for all the cancer subjects was significantly lower than that for their controls (198 and 221 $\mu g l^{-1}$ respectively, $P=0.007$). Following previous practice (Wald *et al.*, 1987), specific cancer sites were analysed separately if 15 or more men had developed cancer at that site. Stomach cancer (13 subjects) was also considered separately but other sites were grouped together. There was no evidence of a differential effect of beta-carotene according to cancer site (a test for heterogeneity between the seven sites being non-significant, $P>0.2$), but, for some of the sites, the number of cancer subjects available for analysis was small and the possibility of different site-specific effects cannot be excluded. The greatest observed effects were for cancer of the lung and stomach.

Table II shows the mean serum beta-carotene of subjects and controls according to the interval between blood collection and diagnosis of cancer and Table III shows, in the same way, the data for lung cancer alone. There was no evidence that the differences in serum beta-carotene between cancer subjects and controls differed for the four periods considered in the two tables. In particular, it appeared that the difference in beta-carotene was present in subjects who had blood collected several years before the diagnosis of cancer. (The mean levels in both cancer subjects and controls decreased with increasing time to diagnosis on account of the concomitant increase in duration of storage of the samples).

Table IV shows the number of subjects and controls and relative risks of cancer according to the quintile of serum beta-carotene concentration. There was a statistically significant inverse trend in relative risk ($P=0.01$); the risk for men in the highest two quintiles of serum beta-carotene was only about 60% of the risk for men in the lowest quintile. Table V, shows in the same way, the data for lung cancer alone.

In the design of our study we matched subjects with controls for age, smoking habits and duration of storage of the serum sample. Mean beta-carotene concentrations classified according to the age of the subject at the time of blood collection showed no consistent pattern (Table VI). Table VII shows the mean serum beta-carotene levels according to smoking category. Smokers had lower beta-carotene levels than non-smokers; the lowest beta-carotene levels tended to occur in the heavier smokers. Table VIII shows the mean beta-carotene levels according to duration of storage of the serum sample. There was a general decline in serum beta-carotene level with increasing storage time; on average the concentration declined by ~5% per year. Therefore, in our analysis matching for smoking habits and duration of storage emerged as being important while matching for age less so.

Discussion

We have shown that, as expected from the dietary studies of

Table I Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in cancer subjects and matched controls according to site of cancer

Cancer site	Numbers of		Mean beta-carotene		Percentage difference[a] (approximate standard error)
	Cancer subjects	Controls	Cancer subjects	Controls	
Lung	50	99	158	203	−22% (8%)
Colorectal	30	59	203	228	−11% (10%)
Stomach	13	26	180	248	−27% (17%)
Bladder	15	29	211	231	−9% (20%)
CNS[b]	17	34	188	208	−10% (15%)
Skin	56	107	226	234	−3% (8%)
Other	90	179	209	218	−4% (6%)
All sites	271	533	198	221	−10% (4%)

[a]Percentage difference = (mean in cancer subjects minus mean in controls)/mean in controls; [b]Central nervous system.

Table II Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in cancer subjects and matched controls according to interval between blood collection and diagnosis of cancer

Time to diagnosis	*Number of*		*Mean beta-carotene*		*Percentage difference*[a] *(approximate standard error)*
	Cancer subjects	*Controls*	*Cancer subjects*	*Controls*	
Before 1 year	90	172	222	256	−13% (7%)
1–2 years	61	121	200	218	−8% (8%)
3–4 years	61	122	185	196	−6% (7%)
5 or more years	59	118	176	202	−13% (8%)
All periods	271	533	198	221	−10% (4%)

[a]Percentage difference = (mean in cancer subjects minus mean in controls)/mean in controls.

Table III Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in lung cancer subjects and matched controls according to interval between blood collection and diagnosis of cancer

Time to diagnosis	*Number of*		*Mean beta-carotene*		*Percentage difference*[a] *(approximate standard error)*
	Cancer subjects	*Controls*	*Cancer subjects*	*Controls*	
Before 1 year	9	17	129	235	−45% (18%)
1–2 years	12	24	209	196	+7% (29%)
3–4 years	12	24	136	199	−32% (16%)
5 or more years	17	34	160	195	−18% (14%)
All periods	50	99	158	203	−22% (8%)

[a]Percentage difference = (mean in cancer subjects minus mean in controls)/mean in controls.

Table IV Relative risk of cancer according to serum beta-carotene concentration

Beta-carotene concentration		*Number of*		
Quintile	*Limits* ($\mu g l^{-1}$)	*Cancer subjects*	*Controls*	*Relative risk*[a]
1st	10–134	64	100	1.33
2nd	135–185	60	99	1.21
3rd	186–248	53	104	1.02
4th	249–350	47	116	0.81
5th	351–978	47	114	0.80
All	10–978	271	533	1.00

[a]Relative risks take into account the matched design of the study and are expressed relative to the risk in the 'all' category. Test for trend: $P = 0.01$.

Table V Relative risk of lung cancer according to serum beta-carotene concentration

Beta-carotene concentration	*Number of*		
Quintile	*Cancer subjects*	*Controls*	*Relative risk*[a]
1st (lowest)	20	18	2.00
2nd	14	27	0.93
3rd	7	19	0.68
4th	4	23	0.35
5th (highest)	5	12	0.82
All	50	99	1.00

[a]Relative risks take into account the matched design of the study and are expressed relative to the risk in the 'all' category. Test for trend: $P = 0.008$.

Table VI Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in cancer subjects and controls according to age at blood collection

Age (years)	*Cancer subjects*		*Controls*		*All*		
	Number of men	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Approximate standard error*
35–39	10	157	23	244	33	201	19
40–44	27	194	64	217	91	210	12
45–49	49	201	83	217	132	210	10
50–54	57	206	121	214	178	211	10
55–59	63	205	140	225	203	219	9
60–64	65	192	102	227	167	213	11

Table VII Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in cancer subjects and controls according to smoking status and stated cigarette consumption at the time of blood collection

	Cancer subjects		*Controls*		*All*[a]		
Smoking category	*Number of men*	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Approximate standard error*
Life-long non-smokers	47	242	93	261	140	255	12
Ex-smokers	88	213	175	235	263	228	8
Smokers of cigarettes alone:							
1–9/day	14	257	19	243	33	249	25
10–19/day	20	172	33	183	53	179	12
20–29/day	19	142	49	168	68	160	11
30 or more/day	25	160	43	198	68	183	14
All	78	172	144	189	222	183	7
Other smokers	58	183	121	212	179	202	8

[a]For differences between four main smoking categories, $P<0.0001$. For a linear trend, within smokers of cigarettes alone, according to stated consumption per day, $P=0.03$.

Table VIII Mean serum beta-carotene concentrations ($\mu g l^{-1}$) in cancer subjects and controls according to duration of storage of the serum sample

	Cancer subjects		*Controls*		*All*[a]		
Storage time (years)	*Number of men*	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Number of men*	*Mean beta-carotene*	*Approximate standard error*
<3	26	283	50	232	76	248	15
3–	24	256	50	259	74	258	15
4–	37	198	66	238	103	223	13
5–	60	196	122	235	182	221	9
6–	38	177	72	204	110	194	11
7–	32	149	66	217	98	192	12
8–	30	203	58	199	88	200	12
≧9	24	185	49	178	73	180	13

[a]For linear trend according to storage time, $P<0.0001$.

Table IX Summary of the prospective biochemical epidemiological studies of beta-carotene and cancer

Study	*Sex of subjects*	*Site of cancer*	*Number of:*		*Approximate mean time to diagnosis of cancer (years)*	*Plasma (P) or serum (S)*	*Overall mean beta-carotene ($\mu g l^{-1}$) (approximate s.d.)*[e]	*Mean beta-carotene difference ($\mu g l^{-1}$). Cancer subjects minus controls (approximate s.d.)*[e]	*Published statistical significance*
			Cancer subjects	*Controls*					
Stähelin *et al.* (1984)	Male	All	115	308	4	P	206 (131)	−55 (14)[d]	not given
Willett *et al.* (1984*a*)	Both	All	111	210	3	S	1,126 (569)[a]	+34 (64)	$P=0.63$[b]
Nomura *et al.* (1985)	Male	5 sites	284	302	5	S	263 (252)[e]	−56 (21)[d]	$P=0.004$[f]
Menkes *et al.* (1986)	Both	Lung	99	196	5	S	278 (234)	−40 (29)	$P=0.001$[b]
Present study	Male	All	271	533	3	S	213 (130)[b]	−23 (9)[b]	$P=0.007$[b]
Overall	—	—	880	1,549	—	—	— —	−35 (7)[c]	$P=0.001$

[a]Total carotenids were assayed, not beta-carotene; [b] Values obtained from analysis of log beta-carotene; [c]The overall mean across studies was calculated as a mean of the individual mean differences, each weighted inversely according to its variance; [d]Mean difference not adjusted for smoking; [e]s.d. based on the 25th and 75th percentiles in controls; [f]P value for linear trend in log odds ratio over quantiles of beta-carotene. (P value adjusted for smoking is given as 0.04).

beta-carotene and cancer, there is an inverse association between serum beta-carotene and the risk of cancer and that the effect is present for five and more years before the diagnosis of the cancer. The study was not large enough to be able to indicate with confidence the sites of cancer for which the association applied though it is of interest that the association was strongest for lung cancer, which is consistent with the prospective dietary studies of beta-carotene intake (Bjelke, 1975; Shekelle *et al.*, 1981; Kvale *et al.*, 1983).

There are four other published prospective studies of serum beta-carotene or total carotenoids and cancer. These are summarised in Table IX together with our present results. Overall the results are consistent in showing that the average serum beta-carotene level was lower in subjects who developed cancer than in those who did not. In one study (Willett *et al.*, 1984), total carotenoids were measured rather than beta-carotene alone and showed no significant difference between cancer subjects and controls. In the reports of two of the other studies (Stähelin *et al.*, 1984 and Nomura *et al.*, 1985) the mean beta-carotene differences between cancer subjects and controls were not adjusted for smoking habit. Since smokers tend to have lower serum

beta-carotene concentrations than non-smokers and smoking is also associated with the risk of cancer the mean differences between cancer subjects and controls given for these two studies is likely to over-estimate the differences in beta-carotene that relate, independently of smoking habit, to the risk of cancer. Nonetheless, the results of the two remaining studies in Table IX (Menkes *et al.*, 1986 and the present study) which allowed for smoking habits showed a similar, but less marked, effect.

The observation that the inverse association between beta-carotene and the risk of cancer persists for several years before the diagnosis of cancer, and does not appear to show a greater effect in those cancer cases for which the time between blood collection and diagnosis was short, indicates that the low beta-carotene level probably precedes the development of cancer. The fact that, in our study, there were only 59 men in whom a diagnosis of cancer was made more than five years after the time of blood collection means that we cannot completely exclude the possibility that early cancer may itself influence serum beta-carotene levels but, taken with the known long-term association between beta-carotene *consumption* and cancer, it is unlikely. The inverse association could arise either directly, because beta-carotene reduces the risk of cancer, or indirectly, because beta-carotene intake is associated with the intake of another dietary component that affects the risk of cancer. Which of the two explanations is the correct one should emerge from the results of the large-scale randomised trial of beta-carotene supplementation currently in progress among physicians in the United States of America (Hennekens, 1986). Whatever the answer the association represents a most interesting epidemiological clue to the link between diet and cancer.

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