The Lancet · Saturday 28 November 1981

DIETARY VITAMIN A AND RISK OF CANCER IN THE WESTERN ELECTRIC STUDY

RICHARD B. SHEKELLE
SHUGUEY LIU
WILLAM J. RAYNOR, JR
MARK LEPPER
CAROL MALIZA
ARTHUR H. ROSSOF

Departments of Preventive Medicine and of Medicine, Rush-Presbyterian-St Luke's Medical Center, Chicago, Illinois U.S.A.

OGLESBY PAUL

Department of Medicine, Harvard Medical School, Boston, Massachusetts

ANNE MACMILLAN SHRYOCK

School of Nursing, University of Michigan, Ann Arbor, Michigan

JEREMIAH STAMLER

Department of Community Health and Preventive Medicine, Northwestern University School of Medicine, Chicago, Illinois

Summary Intake of dietary provitamin A (carotene) was inversely related to the 19-year incidence of lung cancer in a prospective epidemiological study of 1954 middle-aged men. The relative risks of lung cancer in the first (lowest) to fourth quartiles of the distribution of carotene intake were respectively, 7·0, 5·5, 3·0, and 1·0 for all men in the study, and 8·1, 5·6, 3·9, and 1·0 for men who had smoked cigarettes for 30 or more years. Intake of preformed vitamin A (retinol) and intake of other nutrients were not significantly related to the risk of lung cancer. Neither carotene nor retinol intake was significantly related to the risk of other carcinomas grouped together, although for men in whom epidermoid carcinomas of the head and neck subsequently developed, carotene intake tended to be below average. These results support the hypothesis that dietary beta-carotene decreases the risk of lung cancer. However, cigarette smoking also increases the risk of serious diseases other than lung cancer, and there is no evidence that dietary carotenoids affect these other risks in any way.

Introduction

PROSPECTIVE epidemiological investigations in Norway[1] and Japan[2] and case-control studies in Singapore,[3] the U.S.A.,[4] and the U.K.[5] have shown an inverse association between intake of foods rich in vitamin A and risk of lung cancer. Although some results were presented for selected groups of foods, these studies did not clearly determine whether the risk was associated with intake of preformed vitamin A (described here as retinol), intake of provitamin A (compounds that can be converted to vitamin A in the body, described here as carotene), or intake of both. Retinol occurs naturally only in certain foods of animal origin (chiefly whole milk, cheese, butter, egg yolk, and liver), and the principal sources of carotene are dark-green leafy vegetables, carrots, and certain yellow and red fruits and vegetables. Peto et al.[6] have argued in support of the hypothesis that dietary beta-carotene, but not dietary retinol, reduces the risk of cancers in man. This study was undertaken to investigate the association between intake of carotene and retinol in the diet as assessed in 1959, and the risk of cancer during the following 19 years in a group of men participating in a prospective epidemiological study. Our hypothesis, based on the consistent epidemiological evidence on vitamin A and lung cancer and the arguments of Peto et al.,[6] was that intake of dietary carotene would be inversely related to risk of lung cancer.

Subjects and Methods

In 1957 3102 men were randomly selected for the Western Electric Study from 5397 men aged 40–55 years who had been employed for at least 2 years at the Western Electric Company's Hawthorne Works in the Chicago metropolitan area. 2080 (67·1%) of the selected men agreed to participate. Another 27 men served as a pilot group, bringing to 2107 the total number initially examined from October, 1957, to December, 1958. Approximately 65% were first and second generation Americans, predominantly of German, Polish, or Bohemian ancestry. Most of the other men were descendants of earlier emigrants from the British Isles. The men worked at various occupations associated with the manufacture of telephones and related products. Selection, examination, and follow-up procedures have been described elsewhere.[7]

Two nutritionists obtained information about diet at both the initial examination and the second examination 1 year later. They used a standard 1 h interview and a questionnaire to the homemaker to determine the kinds and quantities of foods and beverages consumed during the previous 28 days. The interview asked about the customary pattern of eating on workdays and weekends, and included a detailed review of 195 specific foods to determine how often each had been eaten and the usual size of portions. Models of common foods and dishes of various sizes were used as aids. Questions about food supplements were asked, but the information was not coded or included in assessments of nutrient intake because they were so rarely used. The data were analysed by a food composition table derived from several sources[8-11] to estimate each

participant's usual daily intake of energy and of various nutrients, including total vitamin A—i.e., the sum of retinol and carotene. Re-analysis to estimate carotene intake and retinol intake separately is not now possible because the dietary histories are no longer available. However, at the second examination, in addition to estimation of nutrients, food-profile scores (0–3) were used to indicate consumption of 26 separate foods or food groups formed from the original list of 195 items. The score 0 was always assigned to zero units; other scores were defined by ranges of values. For instance, the food-profile scores for eggs were defined as follows: 0=0, 1=1–11, 2=12–28, and 3=29 or more eggs per 28-day period. Selected food-profile scores were used to estimate the proportional contributions of retinol and carotene to intake of total vitamin A (table I). For this purpose, the range of values that originally defined a food-profile score was replaced by a single characteristic value, shown in parentheses in table I. The amount of vitamin A activity per unit for a group of foods was estimated by averaging the amounts shown in the food composition table for each of the foods in the group. Although some foods in a group were undoubtedly eaten more often than others, data on relative frequency of consumption of foods within a group in this population are not available. Therefore, a simple unweighted mean value was used to avoid the influence of subjective judgment on the results.

The amount of retinol (R) was estimated by multiplying, for each of 8 food groups shown in the upper section of table I, the amount of vitamin A per unit of the food by the number of units consumed, as indicated by the characteristic value of the food-profile score, and summing these products. Carotene (C) was similarly estimated from the 3 food groups shown in the lower section of table I. The proportional contribution of retinol, R/(R+C), was multiplied by the intake of total vitamin A (VITA), estimated by the earlier nutrient analysis of the 28-day diet history taken at the second examination, to calculate the retinol index (RI): RI=VITA [R/(R+C)]. The carotene index (CI) was calculated as CI=VITA−RI.

Men continuing to participate in this study were re-examined annually until 1969. 9 years later, vital status was determined for all but 3 of the 2107 participants. Death certificates were obtained for all those who had died. We sent all the survivors a questionnaire which included questions on history of diagnosis of cancer; replies were obtained from all but 19 of 1546 survivors. Medical and hospital records were sought, with the permission of the participant or his next-of-kin, for all men with malignant neoplasm indicated on the death certificate or the questionnaire (n=285) and were obtained for 243 of these men (85·3%). This information was reviewed and coded without knowledge of other information about participants.

153 men (7·3% of 2107) were omitted from this analysis for one or more of the following reasons: age at first examination was less than 40 years (1 man); vital status was unknown at the twentieth anniversary (3 men); malignant neoplasm had been diagnosed before the second examination (9); the second examination was not conducted owing to death (14), leaving the company's employment or transfer to another plant (17), or withdrawal from the study (31); and data were missing from the second examination on the food-profile scores (74) or cigarette smoking (6).

Data were analysed by the Statistical Analysis System, Release 79·3A.[12] Proportional hazards (Cox-type) regression analysis was performed by PROC PHGLM.[13,14] Univariate tests for linear trends in incidence and mortality rates were conducted as described by Fleiss.[15]

Results

The mean carotene index was 5543±2769 (1 SD) IU/day. The carotene index was inversely related to the incidence of lung cancer (table II) but apparently was not related to the incidence of other carcinomas grouped together. The relative risks of lung cancer in the first (lowest) to the fourth (highest) quartiles of the carotene index were 7·0, 5·5, 3·0, and 1·0, respectively. Food-profile scores for all 3 food groups used to calculate the carotene index tended to be inversely related to the risk of lung cancer, although only the association with the score for soup was significant at the 5% level. The p values for vegetables, soups, and fruit were 0·225, 0·033, and 0·073, respectively. The small number of men (33) in whom lung cancer developed, and the large proportion of them for whom no information about type was available (see footnote to table II), precluded statistical analysis according to type of lung cancer.

The mean retinol index was 4734±3196 (1 SD) IU/day. The simple coefficient of correlation with the carotene index was 0·285, and the partial correlation coefficient was 0·172 after adjustment for total energy intake. The retinol index was not significantly associated with incidence of lung cancer or of other cancers (table II). 4 of the food groups forming the retinol index (whole milk, cream, butter, and cheese) tended to be inversely related to risk of lung cancer, but no relation

TABLE I—UNIT OF MEASUREMENT, VITAMIN A PER UNIT, AND NUMBER OF UNITS INDICATED BY EACH FOOD-PROFILE SCORE FOR FOOD GROUPS FORMING RETINOL AND CAROTENE INDICES

Food groups	Unit of measurement	Vitamin A (IU/food unit)	Number of units indicated by food-profile score		
			1	2	3
Forming the retinol index:					
Whole milk	480 ml	780	1–27 (14)	28 (28)	⩾29 (56)
Cream	30 ml	249	1–13 (7)	14–84 (49)	⩾85 (168)
Butter	14 g	460	1–27 (14)	28–84 (56)	⩾85 (140)
Margarine*	14 g	460	1–27 (14)	28–84 (56)	⩾85 (140)
Cheese†	28 g	400	1– 7 (4)	8–16 (12)	⩾17 (32)
Ice cream, custard, pudding	120 ml	330‡	1– 3 (2)	4–12 (8)	⩾13 (24)
Eggs	54 g	550	1–11 (6)	12–28 (20)	⩾29 (56)
Liver§	120 g	52 680	<1 (0·5)	1–2 (1·5)	⩾ 3 (4)
Forming the carotene index:					
Vegetables	100 g	2560¶	1–27 (9)	28–84 (42)	⩾85 (98)
Soup	240 ml	1113‖	1–11 (3)	12–28 (16)	⩾29 (42)
Fruit	100 g	940**	1–27 (9)	28–84 (42)	⩾85 (98)

Food-profile score 0 was used to indicate 0 units. Scores 1–3 were initially defined by ranges of values; to calculate the retinol and carotene indices, these ranges were replaced by characteristic values (in parentheses). *Margarine was fortified with vitamin A, mostly retinyl esters, to the same level as butter. In the late 1950s, 3·3 mg (5500 IU) β-carotene was added as a colouring agent to each pound (453·6 g) in about one-third of margarines. †Value for American cheese used. ‡Mean value for three items comprising this group was assigned. §Value for beef liver used. ¶Nominal value was obtained by averaging value for each of separate items listed in this group: asparagus, green beans, beets, broccoli, cabbage, carrots, cauliflower, corn, eggplant, leafy green vegetables, other green and yellow vegetables, onions, peas, and tomatoes. ‖ Value for composite soup was used. **Nominal value was obtained by averaging value for each of separate items listed in this group: avocado, apple, banana, cantaloupe, citrus fruit, other fresh or canned fruit, and dried fruit.

TABLE II—19-YEAR RISK OF CARCINOMA ACCORDING TO LEVEL OF DIETARY CAROTENE AND RETINOL INDICES IN 1954 MIDDLE-AGED MEN

Mean dietary variable (range)	No. at risk	Mean age (yr)*	19-yr incidence of carcinoma†					
			Lung‡		Other§		Total	
			No.	(%)	No.	(%)	No.	(%)
Quartiles of carotene index (100 IU/day):								
27 (1–37)	488	48·8	14	(2·9)	42	(8·6)	56	(11·5)
45 (38–50)	489	48·9	11	(2·2)	54	(11·0)	65	(13·3)
58 (51–66)	489	48·5	6	(1·2)	38	(7·8)	44	(9·0)
91 (67–320)	488	48·7	2	(0·4)	41	(8·4)	43	(8·8)
Total 55	1954	48·7	33	(1·7)	175	(9·0)	208	(10·6)
Slope			−0·037		−0·011		−0·048	
χ^2 slope (df=1)			8·688		0·165		2·586	
p			0·003		0·684		0·108	
Quartiles of retinol index (100 IU/day):								
16 (1–22)	488	48·8	5	(1·0)	44	(9·0)	49	(10·0)
32 (23–39)	489	48·5	11	(2·2)	39	(8·0)	50	(10·2)
50 (40–62)	489	48·9	7	(1·4)	48	(9·8)	55	(11·2)
92 (63–255)	488	48·7	10	(2·0)	44	(9·0)	54	(11·1)
Total 47	1954	48·7	33	(1·7)	175	(9·0)	208	(10·6)
Slope			0·009		0·011		0·020	
χ^2 slope (df=1)			0·781		0·214		0·636	
p			0·377		0·644		0·425	

*Age at the second examination. †Rates have not been age-adjusted because age, within the relatively narrow range studied here, was not correlated with the carotene or retinol indices and, consequently, quartiles did not differ substantially in distribution of age. ‡Lung carcinomas: 4 adenocarcinomas; 2 small-cell; 2 large-cell; 9 epidermoid carcinomas; and 16 bronchogenic carcinomas of unspecified type. §Other carcinomas: 36 non-melanoma skin; 29 colon; 29 prostate; 20 rectum; 19 urinary bladder; 14 epidermoid head and neck; 7 kidney; 5 stomach; 5 pancreas; and 11 patients with generalised carcinomatosis with unknown primary site.

was significant; the p values were 0·944, 0·170, 0·798, and 0·824, respectively. The other 4 food groups tended to be positively related to the risk of lung cancer, but only the trend for margarine was significant; the p values for margarine, ice cream/custard/puddings, eggs, and liver were 0·041, 0·190, 0·325, and 0·459, respectively.

The retinol index was not significantly correlated with age, with cigarettes smoked per day, with years of smoking cigarettes, or with serum cholesterol. The carotene index was not significantly correlated with age or with serum cholesterol, but there was a small negative correlation with cigarettes smoked per day ($r = -0·06$) and with years of smoking ($r = -0·06$).

The level of the dietary carotene index and the duration of cigarette smoking were both associated with incidence of lung cancer (fig. 1). Cox-type regression analysis (table III) confirmed that the carotene index had a significant inverse association with incidence of lung cancer after adjustment for duration of cigarette smoking, number of cigarettes smoked per day, the retinol index, and age. Although age and cigarettes smoked per day were significantly and positively associated with risk of lung cancer in univariate analysis (not shown here), these associations were reduced in magnitude and were not significant at the 5% level in multivariate analyses that included duration of smoking.

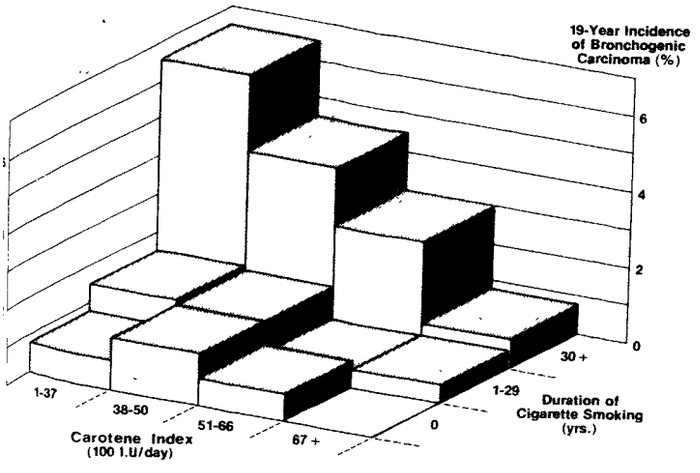

Fig. 1—Bivariate association of carotene index and duration of cigarette smoking with 19-year incidence of lung cancer.

Ratio of cases to number at risk in each quartile of carotene index, low to high, for men who reportedly had never smoked cigarettes was 1/129, 2/139, 1/149, and 0/158; for men smoking 1–29 years, 3/204, 3/218, 1/208, and 1/211; and for men smoking >30 years, 10/155, 6/132, 4/132, and 1/119.

Men in whom lung cancer developed tended to have had below-average intake of dietary carotene at the beginning of the study; this tendency persisted throughout the 19 years of follow-up (fig. 2). Below-median values of carotene intake had been recorded for 11 of 16 men in whom lung cancer was diagnosed during the first 13 years of follow-up and for 14 of 17 men diagnosed during the 14th–19th years. Overall, 14 men (42% of the 33 with lung cancer) had values of carotene intake below the 25th percentile, and 2 (6%) had values above the 75th percentile.

TABLE III—PROPORTIONAL HAZARDS (COX-TYPE) REGRESSION OF 19-YEAR INCIDENCE OF LUNG CANCER ON THE CAROTENE AND RETINOL INDICES, THE DURATION AND AMOUNT OF CIGARETTE SMOKING, AND AGE

Regression of 19-year incidence of lung cancer on:	Coefficient	Standard error	χ^2	p
Duration of cigarette smoking (yr)	0·070	0·021	10·94	<0·001
Carotene index (100 IU/day)	−0·024	0·009	7·78	0·005
Retinol index (100 IU/day)	0·009	0·005	3·02	0·082
Age at 2nd examination (yr)	0·079	0·046	2·89	0·089
Number of cigarettes smoked per day most of adult life	−0·012	0·023	0·29	0·590

Results for a total of 1954 men, in 33 of whom lung cancer subsequently developed, were analysed. χ^2 for the overall model with 5 variables was 37·82, which with 5 degrees of freedom is associated with $p<0·001$.

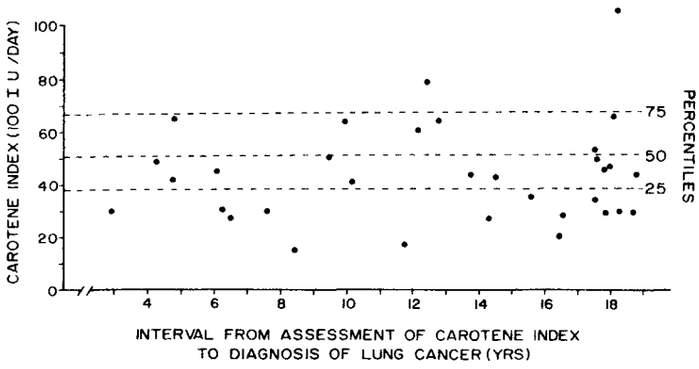

Fig. 2—Values of dietary carotene index for 33 men in whom bronchogenic carcinoma subsequently developed according to time from dietary assessment to dignosis of cancer.

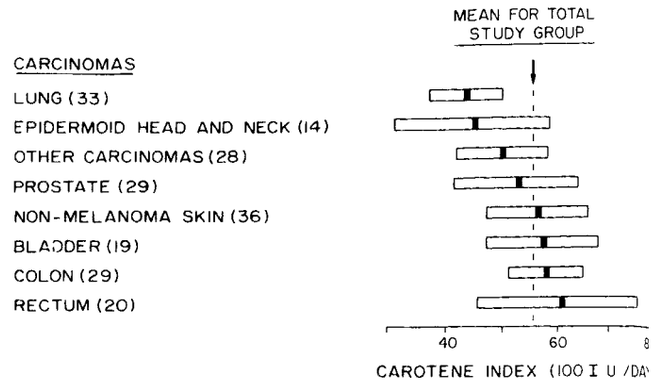

Fig. 3—Mean value of the dietary carotene index and its 95% confidence interval for each of 8 categories of carcinoma which occurred in a group of 1954 men followed for 19 years.

Number of men in each category is shown in parentheses. Group of 28 other carcinomas distributed by site as follows: 7 kidney, 5 stomach, 5 pancreas, and 11 cases of generalised carcinomatosis with primary site unknown. Broken line = mean value for total group of 1954 men; solid bar = mean carotene index; open bar = 95% confidence interval.

Fig. 3 shows the mean carotene index, and its 95% confidence interval, for each of 8 categories of carcinomas compared with the mean value for the entire study group of 1954 men. Although the mean carotene index for the men in whom epidermoid carcinomas of the head and neck developed was only slightly higher than that for the men in whom lung cancer developed (4481 *vs* 4342 IU/day), the 95% confidence interval was substantially larger and encompassed the mean value for the total study group owing to a larger standard deviation (2642 *vs* 1886 IU/day) and a smaller number of men. None of the remaining categories of carcinoma had a mean carotene index significantly different from the overall mean value.

The mean intake of dietary carotene was lower in men who subsequently developed lung cancer than in men who did not, but mean intake of retinol, mean energy intake, and mean intake of other nutrients were similar in the two groups (table IV).

The level of the carotene index was significantly inversely related to 19-year mortality from lung cancer but not from other carcinomas, other malignant neoplasms, cardiovascular-renal diseases, or other causes grouped together (table V). In contrast, cigarette-smoking status was strongly associated with risk of death in every category of cause except malignant neoplasms other than carcinomas.

Discussion

Both evidence from animal experimental studies and the epidemiological evidence on vitamin A and risk of cancer have been reviewed in detail.[6,16] The results of our study support the hypothesis of Peto et al. with respect to lung cancer; the dietary variable related to risk of lung cancer is beta-carotene, not retinol. There were no significant differences in mean intake of other nutrients by men in whom lung cancer developed and by those in whom it did not during 19 years of follow-up; this strengthens the view that the risk of lung cancer was specifically related to intake of carotene and not to some other variable associated with eating fruits and vegetables. The long period of follow-up indicates that below-average intake of carotene preceded the carcinoma and was not a consequence of it.

All dietary survey methods have problems with reliability and validity,[17] but the methods for assessing intake of vitamin A in previous epidemiological studies (excepting Gregor's[5]) and of carotene and retinol in our study, were particularly

TABLE IV—DIFFERENCES IN MEAN VALUE OF NUTRITIONAL VARIABLES AT START OF STUDY BETWEEN MEN WHO SUBSEQUENTLY DEVELOPED LUNG CANCER AND THOSE WHO DID NOT DURING 19 YEARS OF FOLLOW-UP

Dietary variables (units/day)*	Mean value of nutritional variables ±SD		Difference between means	Student's *t*	p
	Men with lung cancer (n=33)	All others (n=1921)			
Carotene index (100 IU)	43·4±18·9	55·6±27·8	−12·2	−3·65†	<0·001
Retinol index (100 IU)	52·2±34·5	47·3±31·9	4·9	0·88	0·378
Energy intake (100 kcal)	29·5± 8·7	31·1± 8·4	−1·6	−1·10	0·272
Animal protein (% cal)	11·5± 2·1	11·6± 2·1	−0·1	−0·08	0·933
Vegetable protein (% cal)	3·3± 0·5	3·5± 0·6	−0·2	−1·63	0·102
Animal fat (% cal)	32·9± 5·8	32·7± 5·0	0·2	0·21	0·836
Vegetable fat (% cal)	10·4± 3·9	10·2± 3·7	0·2	0·28	0·781
Carbohydrate (% cal)	36·5± 5·6	38·0± 4·7	−1·5	−1·83	0·067
Calcium (g)	1·02± 0·46	0·92±0·34	0·10	1·63†	0·112
Phosphorus (g)	2·35±0·86	2·18±0·85	0·17	1·13	0·260
Iron (mg)	15·8± 5·4	16·8± 4·4	−1·0	−1·33	0·184
Thiamin (mg)	1·52±0·52	1·63±0·47	−0·11	−1·33	0·185
Riboflavin (mg)	2·26±0·70	2·41±0·78	−0·15	−1·11	0·266
Niacin (mg)	21·0± 6·5	22·8± 6·2	−1·8	−1·63	0·104
Vitamin C (mg)	91·8±31·4	101·0±41·1	−9·2	−1·28	0·200
Vitamin D (IU)	175±117	179±132	−4·0	−0·19	0·848
Cholesterol (mg)	774±472	729±249	45	0·55	0·586

*Carotene and retinol indices were measured only at the second examination. Values of other dietary variables were obtained by averaging measurements made at both the first and second examinations to decrease the effect of intra-individual variation. †For these variables the value of Student's *t* and its degrees of freedom were calculated by the Statistical Analysis System approximation in the case of unequal variances.

TABLE V—19-YEAR MORTALITY RATE PER 100 ACCORDING TO LEVEL OF THE CAROTENE INDEX AND TO CIGARETTE SMOKING STATUS

—	No. at risk	Percentage dead by cause					
		Lung cancer	Other cancers	Other malignant neoplasms	Cardio-vascular/ renal diseases	Other causes	Total
Quartiles of carotene index (100 IU/day):							
1–37	488	2·7	3·7	1·0	16·0	3·3	26·6
38–50	489	1·8	4·3	1·4	13·9	2·2	23·7
51–66	489	0·6	2·9	1·2	16·4	3·7	24·7
67–320	488	0·2	3·1	0·8	13·7	2·9	20·7
Slope		−0·036	−0·012	−0·004	−0·019	0·000	−0·071
χ^2 slope (df = 1)		10·827	0·441	0·176	0·300	0·000	2·993
p		0·001	0·507	0·675	0·584	1·000	0·084
Cigarette smoking:							
Never	575	0·5	2·4	0·9	10·8	1·2	15·8
Former	266	0·0	2·6	1·5	10·5	4·9	19·5
Current, 1–14/day	279	1·1	3·6	2·1	13·6	2·5	22·9
Current, 15–24/day	644	2·3	3·9	0·8	19·6	3·6	30·1
Current, ⩾25/day	190	2·6	6·3	1·0	20·5	4·7	35·3
Total	1954	1·3	3·5	1·1	15·0	3·0	24·0
Slope		0·082	0·118	0·007	0·478	0·103	0·788
χ^2 slope (df = 1)		10·519	8·429	0·103	36·675	7·369	69·741
p		0·001	0·004	0·749	<0·001	0·007	<0·001

Total number of deaths in each group were: 26 lung cancers, 68 other cancers, 22 other malignant neoplasms, 293 cardiovascular/renal diseases, and 59 other causes. Mean ages for quartiles of carotene index are shown in table II. For five categories of cigarette smoking (never to ⩾25/day) mean ages were 49·0, 49·3, 49·1, 48·2, and 48·1 years, respectively. Rates have not been age-adjusted because age, within relatively narrow range studied here, was not correlated with carotene index and had only a very small negative correlation with quantity of cigarettes smoked. Consequently, the categories did not differ substantially in distribution of age.

crude. It is not possible, therefore, to estimate whether the values of dietary carotene reported here are higher or lower than the values that would be obtained by chemical analysis of duplicate meals. We believe that the correlation between our estimates of carotene intake and the true values, if they were known, would be moderate at best and that these results should be interpreted with considerable caution.

Other studies[18,19] have shown positive correlations between serum cholesterol concentrations and serum levels of retinol and of beta-carotene. Kark et al.[20] suggested that the inverse relation between serum cholesterol level and risk of cancer found in some populations may be secondary to an inverse relation between serum retinol and risk of cancer coupled with a positive correlation between serum retinol and serum cholesterol levels. Our study showed no association between the level of serum cholesterol and the estimated intake of dietary retinol and carotene. However, this finding should also be interpreted cautiously in view of the known difficulties in correlating dietary variables with serum cholesterol concentration.[21,22]

Doll and Peto[23] have shown that the risk of lung cancer depends much more strongly on the number of years that a person has smoked cigarettes than on either chronological age or the number of cigarettes smoked per day. Our results are consistent with their findings.

Many questions remain to be answered, and further studies are required to determine whether increasing the intake of dietary beta-carotene will reduce the risk of lung cancer in man. However, it seems prudent to emphasise that sound nutritional practice, at least for the general populations of countries such as the U.S.A., involves selecting foods from each of several major groups, including the vegetables and fruits that contain substantial amounts of beta-carotene. The consistency of the epidemiological evidence from diverse populations, the graded nature and temporal sequence of the association, its independence from cigarette smoking, and its coherence with the evidence from animals, all suggest that a diet relatively high in beta-carotene may reduce risk of lung cancer even among persons who have smoked cigarettes for many years. It should be emphasised, however, that cigarette smoking increases the risk of other serious diseases, and there is no evidence that dietary carotene affects these other risks in any way.

We thank the officers and employees of the Western Electric Company, in particular, the participants and their families, and all the physicians who were involved in the Western Electric Health Study. We also thank Dr Martha Trulson and Ms Dorothea Turner who helped to prepare the food composition table, Dr Harlley McKean and Mr Daniel Garside who helped to organise the data file, Mr Joseph Costello, Mrs Nancy O'Dell, and Mrs Dolores Vogel who assisted in determining the vital status of participants, and Ms Loralei LeGrady who helped to prepare data tables and manuscript. Dr Charles Hennekens and Mr Richard Peto made helpful suggestions on analysis of data. The Western Electric Study has been supported by the American Heart Association; Mrs Tiffany Blake; Chicago Heart Association; Illini Foundation; Illinois Heart Association; National Heart, Lung and Blood Institute; Research and Education Committee of Presbyterian-St Luke's Hospital; Otho S. Sprague Foundation, and other private donors. This investigation was supported by the Day Fund, the Rush Cancer Center, and the National Cancer Institute (CA 22536).

Correspondence should be addressed to R. B. S., Department of Preventive Medicine, Rush-Presbyterian-St Luke's Medical Center, Chicago, Illinois 60612, U.S.A.

REFERENCES

1. Bjelke E. Dietary vitamin A and human lung cancer. *Int J Cancer* 1975; **15:** 561–65.
2. Hirayama T. Diet and cancer. *Nutr Cancer* 1979; **1:** 67–81.
3. MacLennan R, DaCosta J, Day NE, Low CH, Ng YK, Shanmugaratnam K. Risk factors for lung cancer in Singapore Chinese, a population with high female incidence rates. *Int J Cancer* 1977; **20:** 854–60
4. Mettlin C, Graham S, Swanson M. Vitamin A and lung cancer. *J Natl Cancer Inst* 1979; **62:** 1435–38.
5. Gregor A, Lee PN, Roe FJC, Wilson MJ, Melton A. Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A. *Nutr Cancer* 1980; **2:** 93–97
6. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? *Nature* 1981; **290:** 201–08.
7. Paul O, Lepper MH, Phelan WH, et al. A longitudinal study of coronary heart disease. *Circulation* 1963; **28:** 20–31.
8. Department of Nutrition. Food Composition Table No. 3. Boston: Harvard School of Public Health, 1957.
9. Hayes OB, Rose G Supplementary Food Composition Table. *J Am Diet Assoc* 1957; **33:** 26–29.
10. Hardinge MG, Crooks H. Fatty acid composition of food fats. *J Am Diet Assoc* 1958, **34:** 1065–71.

References continued overleaf

EFFECTS OF 11-WEEK INCREASE IN DIETARY EICOSAPENTAENOIC ACID ON BLEEDING TIME, LIPIDS, AND PLATELET AGGREGATION

MARGARETA THORNGREN ANDERS GUSTAFSON

Medical Department, University Hospital, S-221 85 Lund, Sweden

Summary The effect of a diet rich in eicosapentaenoic acid (EPA) on platelet phospholipid fatty acid composition, platelet aggregation, and bleeding time was studied in 10 healthy men, whose usual diet was partly replaced by fish for 11 weeks. This diet provided 2–3 g EPA per day. Two doses (3·5 and 10 mg/kg body-weight) of acetylsalicylic acid (ASA) were given before and during the diet. The fish diet prolonged bleeding time (by 42%) and decreased platelet aggregability. The changes in platelet phospholipid fatty acid composition consisted of increases in the ω-3 series (C20:5 and C22:6) and decreases in the ω-6 series (C18:2 and C20:3). The reduction in platelet aggregation induced by collagen and ADP did not parallel the changes in platelet membrane phospholipids and bleeding times. Diminished platelet aggregation induced by collagen lasted only 3 weeks (while subject was still on the diet), whereas the decreased sensitivity to ADP persisted for at least 11 weeks after the volunteers had resumed their normal diet. ASA taken before the diet prolonged bleeding time by as much as did the diet itself. ASA taken during the diet prolonged bleeding time by more than the sum of the increases in bleeding time caused by ASA and by the EPA diet separately, but the synergism was not significantly more than additive. The findings suggest that a diet rich in ω-3 polyunsatured fatty acids reduces the interaction between platelets and the vessel wall by mechanisms which are more complex than just a reduction in susceptibility of platelets to the naturally occurring agents collagen and ADP, or an imbalance between proaggregatory and anti-aggregatory prostaglandin derivatives.

Introduction

EPIDEMIOLOGICAL studies have shown that atherosclerotic cardiovascular disease is uncommon among Greenland Eskimos.[1,2] These Eskimos have lower plasma cholesterol and beta-lipoproteins, and much lower plasma triglycerides, than do mainland Danes.[3] These differences are due to differences in diets. The Eskimos also have high plasma levels of eicosapentaenoic acid (EPA), the source of which is the diet.[4-6]

These observations have led to a hypothesis which proposes that large quantities of ω-3 polyunsaturated fatty acids, mainly EPA, in the diet protects Eskimos against the thromboembolic complications of cardiovascular diseases by reducing platelet aggregability.[6]

Various mechanisms have been proposed to account for the decreased platelet aggregability of people on such diets. It has been suggested that EPA can be used by vessel walls to make prostaglandin I_3, which is as potent as prostaglandin I_2 (prostacyclin) in inhibiting platelets; whereas the platelets themselves would produce thromboxane A_3 which, unlike thromboxane A_2, has no aggregating effect.[6] The anti-thrombotic condition brought about by such diets would then be due to an imbalance between opposing prostaglandin derivatives, with the platelet inhibitory effect predominant.

According to the same hypothesis, long-term dietary enrichment with EPA should prolong bleeding time because of reduced platelet aggregability which, in turn, should be related to changes in the fatty acid composition of platelets and plasma lipids.

To elucidate further the possible connections between diet and haemostasis which could explain the low incidence of thrombotic disease in Eskimos, a study was conducted in Lund during the winter of 1980–81. This investigation differs from previous ones, which either consisted of epidemiological surveys of Greenland Eskimos[2,7] or studied the effects of short-term dietary changes in volunteers.[8]

Methods

Subjects

The subjects were ten apparently healthy male volunteers, non-smokers, who were students in Lund and were aged 28–35 years. They had not taken drugs in the 2 months before the study, nor did they take any during the investigation. For the study they took their usual Swedish diet, the only changes being abstinence from alcohol for the 3 days before blood samples were taken and a predominance of fish, mainly mackerel and salmon, in the diet for 11 weeks. This diet was well tolerated. It increased EPA (C20:5, ω-3) intake by 2–3 g per day.

Blood samples were taken at the following times: on two occasions 2 weeks apart during the baseline, pre-diet period (values shown in the figures are those of the first sample); after 3, 6, and 11 weeks of the diet; and after 6 and 11 weeks in the post-diet period. Samples were taken in the morning after an overnight fast of 12 h. In the baseline period the first sampling was done from both left and right arms in order to check whether bleeding time, platelet aggregation, and lipid composition would be affected by technical variability. There were no significant differences between measurements of samples from the two arms except for platelet aggregation, for which maximum optical density change induced by collagen (4 μg/ml) was 18% higher for samples from the right arm than those for the left.

Lipid Analyses

Platelets were prepared for lipid analyses according to a method already described,[9] with minor modifications. Lipids were extracted with chloroform-methanol 2:1 in the presence of butylated hydroxytoluene. Platelet phospholipids were separated by thin-layer chromatography on 0·5 mm silica gel with

PROFESSOR SHEKELLE AND OTHERS: REFERENCES—*continued*

11 Bowes A de P, Church CF Food values of portions commonly used. Philadelphia: JB Lippincott, 1956
12 Helwig JT, Council KA, eds SAS User's Guide. Raleigh, North Carolina. SAS Institute, 1979
13 Kalbfleisch JD, Prentice RL The statistical analysis of failure time data New York. John Wiley and Sons, 1980
14. Harrell F. The PHGLM Procedure In Reinhardt PS, ed. The SAS supplemental library user's guide, 1980 edition Cary, North Carolina: SAS Institute, 1980: 119–31
15 Fleiss JL Statistical methods for rates and proportions 2nd ed New York John Wiley & Sons, 1981: 143–46
16 Sporn MB, Newton DL Chemoprevention of cancer with retinoids *Federation Proc* 1979; **38:** 2528–34
17 Keys A Dietary survey methods in studies on cardiovascular epidemiology. *Voeding* 1965; **26:** 464–83
18. Kark JD, Smith AH, Switzer BR, Hames CG Retinol, carotene, and the cancer/cholesterol association *Lancet* 1981, i 1371
19. Smith AH, Hoggard BM Retinol, carotene, and the cancer/cholesterol association. *Lancet* 1981, i. 1371–72.
20 Kark JD, Smith AH, Hames CG The relationship of serum cholesterol to the incidence of cancer in Evans County, Georgia *J Chron Dis* 1980, **33:** 311–22.
21 Jacobs DR Jr, Anderson JT, Blackburn H Diet and serum cholesterol do zero correlations negate the relationship? *Am J Epidemiol* 1979, **110:** 77–87
22 Liu K, Stamler J, Dyer A, McKeever J, McKeever P Statistical methods to assess and minimize the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol *J Chron Dis* 1978, **31:** 399–418
23 Doll R, Peto R Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong nonsmokers. *J Epidemiol Commun Hlth* 1978, **32:** 303–13