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DETERMINANTS OF PLASMA LEVELS OF BETA-CAROTENE AND RETINOL

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The associations of retinol and beta-carotene plasma concentrations with eight personal variables and the use of seven classes of cardiovascular drugs were studied in over 1,750 patients with nonmelanoma skin cancer enrolled at four American study centers in a cancer prevention clinical trial. Dietary carotene and female sex were positively related to beta-carotene levels, while cigarette smoking and Quetelet index were negatively related. Use of vitamins, beta blockers, or other antihypertensive drugs were also related to beta-carotene levels, but were associated with much smaller changes in these levels. Age and use of other types of cardiovascular drugs were not associated with beta-carotene levels to a statistically significant extent. There was no statistically significant interaction of smoking and dietary carotene in predicting plasma beta-carotene levels. The multiple correlation coefficient between log plasma beta-carotene and the full model was $R = 0.50$. Retinol levels were positively related to male sex and use of vitamins, diuretics, beta blockers, other cardiovascular drugs, and menopausal estrogens, and negatively related to current cigarette smoking and use of nitrates. The multiple correlation coefficient between plasma retinol and the full model was $R = 0.33$. These findings confirm the importance of several previously reported predictors of plasma retinol and beta-carotene levels. They also identify several new predictors of these micronutrient levels.

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Many dietary and biochemical epidemiologic studies have shown an inverse association between beta-carotene and the risk of cancer (1–8). These findings have in-

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creased interest in factors influencing beta-carotene levels in human plasma or serum. Previous studies have indicated that persons tend to have higher levels if their dietary histories suggest greater consumption of green or yellow leafy vegetables containing carotene (9, 10), consistent with the known effects of carotene ingestion on beta-carotene levels (11–13). Women also appear to have higher levels than men (10, 14–16), while smokers (9, 10, 14, 16–18) and ethanol drinkers (9, 10, 14) have lower beta-carotene levels. However, the relation between beta-carotene levels and age (9, 14–16), obesity (9, 10), vitamin use (9, 16), blood lipids (9, 10, 17), and medication use (16) is less clear because the evidence is either scanty or conflicting.

Higher levels of serum retinol (vitamin A) may be associated with a decreased risk of cancer, although the epidemiologic evidence is less consistent than that for beta-carotene (1–5, 8). It appears that lower retinol levels may be a consequence rather than a cause of invasive cancer (8). The relation between personal factors and plasma retinol levels is complex, since hepatic secretion of retinol binding protein maintains fairly constant levels of retinol over a wide range of dietary retinol intake (13, 19, 20). The only factors which are clearly associated with higher retinol levels in humans are male sex (10, 16) and oral contraceptive use (21).

As part of a large multicenter clinical trial of skin cancer prevention with beta-carotene, we obtained information on personal characteristics and plasma retinol and beta-carotene levels from 1,761 participants enrolled at study sites at four American university medical centers. This large sample size, together with sensitive and specific measurements of retinol and beta-carotene levels, permits us to elucidate relations between personal characteristics or medication use and circulating levels of these substances.

Methods

Adults under age 85 years were eligible for enrollment in the clinical trial if they had had at least one histologically confirmed basal or squamous cell skin cancer totally excised after January 1, 1980, and if female, were without childbearing potential. We excluded persons who had clinical hypercarotenemia, active cancer, xeroderma pigmentosum, basal cell nevus syndrome, severe cardiovascular disease, chronic renal failure, active liver disease, malabsorption syndrome, and those who required immunosuppressive therapy or renal dialysis. We also did not enroll vegan vegetarians (those who eat no meat, milk, or eggs), nor participants in any prior cancer prevention protocol. The study randomized a total of 1,805 subjects distributed between Hanover, New Hampshire (437); Los Angeles, California (344); San Francisco, California (303); and Minneapolis, Minnesota (721).

Before distribution of any beta-carotene capsules to patients, 20 ml of venous blood was collected into heparanized Vacutainer tubes (Becton-Dickinson and Company, Rutherford, NJ). After centrifugation, plasma was transferred to polypropylene freezer tubes and promptly stored at −20 to −85 C for up to three months, until shipped on dry ice to the central laboratory facility for storage at −75 C. Freezer tubes were thawed slowly to room temperature, and 0.5 ml aliquots of plasma removed. A separate high-performance liquid chromatographic assay was used for each ana-

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lyte (22, 23). These assays were sensitive (retinol < 10 ng/ml, beta-carotene < 10 ng/ml), specific, and precise (within-day coefficient of variation 2.5 per cent for retinol and 2.3 per cent for beta-carotene). Accuracy of these assays is assessed by our participation in a voluntary quality control program administered by the National Bureau of Standards; our results on reference samples deviated by 20 per cent or more from the "assigned value" in zero of 22 beta-carotene assays and two of 22 retinol assays since October 1986. The stability of both retinol and beta-carotene in our frozen plasma samples using our assays has also been verified (22–24).

Each study participant completed a self-administered questionnaire before randomization. Included were questions on past and current cigarette smoking and frequency of consumption during the previous month of ten fruits and vegetables (e.g., carrots, spinach/kale, broccoli, cantaloupe, etc.), all containing large amounts of carotene. The food frequency questions were originally designed to detect major changes over time in the dietary pattern of study participants, but for purposes of the present analysis, we used published information on usual portion size (25) and the carotene content of these foods (26) to approximate average weekly carotene intake. The study clinical staff determined the height and weight of each participant.

Questions regarding medication use were included. We considered the following seven drug groups: diuretics, beta blockers, antihypertensive medications (other than diuretics, beta blockers, or calcium channel blockers), nitrates, digoxin, other cardiac medications (including calcium channel blockers, antiarrhythmics, etc.), and estrogens (oral or topical). These groups of cardiovascular medications were chosen because of a prior report that use of antihypertensive medications was associated with lower beta-carotene levels (16).

We used multiple regression analysis (27) to study the relation between plasma beta-carotene or plasma retinol levels and age, sex, cigarette smoking, current use of vitamins, dietary carotene intake (mg/day), Quetelet index [weight (kg)/height^2 (m)], geographic location, and season of blood drawing. The distributions of both plasma beta-carotene and dietary carotene intake were nearly symmetric on a logarithmic scale, and their log transformed values were used in analyses. All main effects and first-order interactions of the predictors with sex and current smoking status were included in an initial model. A likelihood ratio F test was used to assess the significance of the interaction terms as a group. If this was significant, a simultaneous test procedure was used to eliminate subsets of the first-order interactions to simplify the model (28). This procedure permits the screening of effects in large models without increasing the overall size of the test. The adjusted regression coefficients and their 95 per cent confidence intervals (CI) for log plasma beta-carotene were transformed to the original scale to give adjusted proportional changes and confidence intervals for geometric mean beta-carotene levels. The statistical package SAS using the procedure GLM was used for the analysis (29). Statistical tests were performed at the $p = 0.05$ level of significance unless otherwise stated.

In separate multiple regression analyses we also examined the relation between plasma levels of retinol or beta-carotene and the use of cardiovascular drugs and menopausal estrogens. The approach used was identical to that described above, with adjustment for all listed personal characteristics and exclusion of nonsignificant interaction terms.

Results

Adequate blood samples were available to determine 1,761 retinol levels and 1,758 beta-carotene levels. Thus, the population considered in this report consisted of 1,223 men (69 per cent) and 538 women (31 per cent). Their ages ranged between 27 and 84 years with a median age of 64 years. At the time of enrollment 40 per cent were taking

vitamins of some sort. The mean Quetelet index (± standard deviation) was 25.1 (± 3.6), with a range of 14.6 to 55.8. Approximately 19 per cent were currently smokers.

The frequency distribution of the plasma beta-carotene levels is shown in figure 1; it was skewed toward higher values but was close to symmetric on the log scale. The mean plasma beta-carotene level was 225 (± 185) ng/ml, and the geometric mean 174 ng/ml, with a 95 per cent CI of 168–180. Table 1 shows the geometric mean plasma beta-carotene levels for each category of the personal characteristics. Levels were higher in women, nonsmokers, lean patients, and those who ate more dietary carotene or took vitamins daily. Higher levels were also found in subjects whose blood samples were drawn in the summer (202 ng/ml) and fall (184 ng/ml) than those drawn in the winter (165 ng/ml) or spring (154 ng/ml).

After adjustment for all the personal characteristics shown in table 1, all factors except age were statistically significant and highly associated with plasma beta-carotene levels. There was also a significant center-season interaction ($p < 0.001$), with slightly different seasonal trends at different study centers. These trends are not shown in table 1, but the reported effects have been adjusted for the center-season interaction. The multiple correlation coefficient between log plasma beta-carotene and the personal characteristics was $R = 0.48$.

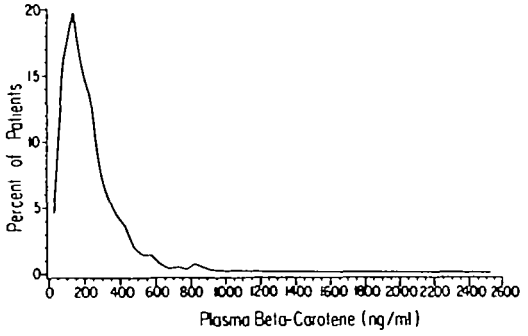

FIGURE 1. Distribution of plasma beta-carotene levels in study patients.

We specifically tested whether there was a statistically significant interaction between dietary carotene and current smoking in the model, since a previous study had indicated that beta-carotene levels of smokers are less affected by dietary intake (10). In our data there was no significant interaction ($p = 0.6$), and the increase in plasma beta-carotene levels with increased dietary intake was similar for both smokers and nonsmokers (figure 2). The regression coefficients (± standard error) of log plasma beta-carotene on log dietary carotene intake, adjusted for the other predictors, were 0.32 (± 0.08) for smokers and 0.37 (± 0.04) for nonsmokers.

Table 2 shows the geometric mean beta-carotene levels according to drug use. After adjustment for personal characteristics and the use of other drug classes, only use of beta blockers and other antihypertensive drugs (not including diuretics, beta blockers, or calcium entry blockers) were statistically significantly associated with beta-carotene levels in plasma. The effect of use of these drugs in the full model was relatively small (9 per cent lower plasma beta-carotene level in beta blocker users, and 14 per cent lower in antihypertensive users). Current use of diuretics, nitrates, digoxin, and estrogens was associated with lower beta-carotene levels as well, though the differences were not statistically significant. The multiple correlation coefficient between log plasma carotene and the full model including drug use increased minimally to $R = 0.50$ from the $R = 0.48$ obtained in the model that did not include drug use.

The frequency distribution of the plasma retinol levels is shown in figure 3. The mean (± standard deviation) was 701 (± 172) ng/ml, with a median of 681 ng/ml. Table 3 shows the mean plasma retinol levels by categories of the personal characteristics, while table 4 shows the effects of various drug classes on retinol levels.

After adjustment for the other personal characteristics, the factors significantly associated with plasma retinol levels were

TABLE 1

Geometric mean plasma beta-carotene levels (ng/ml) and adjusted proportional change in geometric mean plasma carotene levels according to personal characteristics

	n	Geometric mean*	Adjusted proportional change (95% CI)†	*p* value‡
Sex				
Male	1,223	156	1.00 (reference)	<0.001
Female	538	221	1.27 (1.18–1.36)	
Age (years)				
< 40	45	133	1.01 (0.97–1.04)	0.758
40–49	124	164	per decade	
50–59	359	167		
60–69	772	174		
70–79	406	186		
≥ 80	55	183		
Smoking status				
Never smoker	634	201	0.80 (0.76–0.85)	<0.001
Former smoker	797	177	per pack of	
Current smoker (cigarettes/day)			20 cigarettes	
1–19	56	171	currently	
20–39	168	128	smoked	
≥ 40	102	103		
Quetelet index (kg/m^2)				
< 21.0	175	218	0.92 (0.90–0.94)	<0.001
21.0–22.9	288	203	per two units	
23.0–24.9	437	197		
25.0–26.9	377	161		
27.0–28.9	236	148		
≥ 29.0	210	125		
Dietary carotene (mg/day)				
< 1.7	288	130	1.29 (1.23–1.35)	<0.001
1.7–3.4	681	156	for every	
3.5–6.4	527	194	doubling of	
> 6.4	262	254	intake	
Vitamin use				
None	998	164	1.00 (reference)	
Occasional	273	173	0.99 (0.91–1.08)	0.764
Daily	423	196	1.09 (1.01–1.17)	0.030

* Univariate analysis.

† Proportional change (95 per cent confidence interval (CI)) adjusted for the center-season interaction and all other personal characteristics.

‡ For test of significance of adjusted proportional changes.

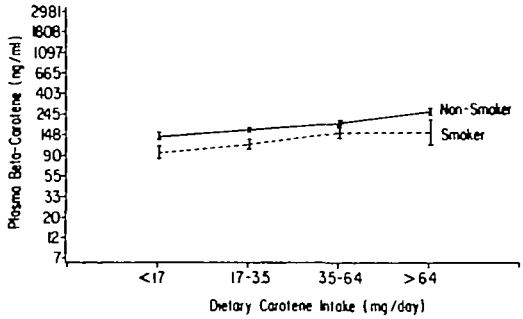

FIGURE 2. Plasma beta-carotene geometric means and 95 per cent confidence intervals by dietary carotene intake and smoking status.

sex, packs of cigarettes currently smoked, and daily vitamin-taking. Women and heavy smokers had lower levels while daily vitamin users had higher plasma retinol levels. Age, Quetelet index, season and dietary carotene intake had no effect on retinol levels. In the full model, retinol levels of former smokers were 9.7 (± 9.2) ng/ml higher than retinol levels of those who had never smoked ($p = 0.3$), while retinol levels of current smokers were 31.3 (± 11.9) ng/ml lower than retinol levels of those who had never smoked ($p < 0.01$) (not shown

TABLE 2

Geometric mean plasma beta-carotene levels (ng/ml) and adjusted proportional change in geometric mean plasma carotene levels according to medication use

	n	Geometric mean*	Adjusted proportional change (95% CI)†	p value‡
Diuretics				
None	1,322	180	1.00 (reference)	
Current	427	156	0.93 (0.86–1.01)	0.083
Beta blockers				
None	1,516	178	1.00 (reference)	
Current	233	145	0.91 (0.82–0.99)	0.047
Other antihypertensives§				
None	1,620	176	1.00 (reference)	
Current	129	146	0.86 (0.75–0.97)	0.016
Nitrates				
None	1,667	175	1.00 (reference)	
Current	82	145	0.92 (0.78–1.08)	0.286
Digoxin				
None	1,670	174	1.00 (reference)	
Current	79	165	0.93 (0.80–1.08)	0.358
Other cardiac drugs§				
None	1,695	174	1.00 (reference)	
Current	54	158	1.03 (0.84–1.25)	0.795
Menopausal estrogens (women only)				
None	464	221	1.00 (reference)	
Current	70	212	0.92 (0.78–1.08)	0.309

* Univariate analysis.

† Proportional change (95 per cent confidence interval (CI)) adjusted for the center-season interaction, the personal characteristics, and all other drug use.

‡ For test of significance of adjusted proportional changes.

§ See text for details.

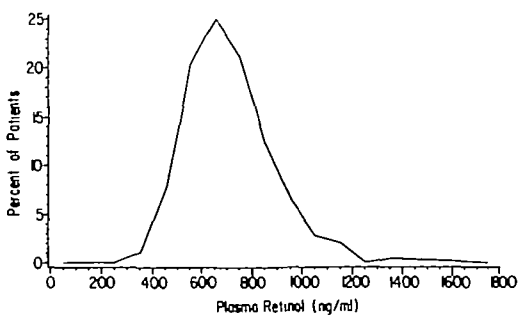

FIGURE 3. Distribution of plasma retinol levels in study patients.

in table 3). There was no evidence of a statistically significant interaction of any of the predictors with either sex or current smoking status. The multiple correlation coefficient between plasma retinol and the personal characteristics was $R = 0.26$.

In an analysis which adjusted for personal characteristics and the use of other drug classes, the medications that were associated with higher retinol levels were diuretics, beta blockers, other cardiovascular drugs, and menopausal estrogens. Use of nitrates was associated with a moderate decrease in retinol levels. The multiple correlation coefficient between plasma retinol and the full model including drug effects increased from $R = 0.26$ to $R = 0.33$.

There was no association between plasma levels of beta-carotene and retinol ($r = 0.04$).

DISCUSSION

In this cross-sectional study of over 1,750 patients with previous nonmelanoma skin cancers, we found mean beta-carotene levels similar to those observed previously (5, 10, 16, 30). Our data confirm earlier reports of positive relations between beta-carotene levels and dietary carotene, female sex, and vitamin use, and a negative relation with

TABLE 3

Mean plasma retinol level (ng/ml) and adjusted regression coefficients according to personal characteristics

	n	Mean*	Adjusted regression coefficient (SE)†	*p* value‡
Sex				
Male	1,222	719		
Female	536	660	−56.6 (9.2)	<0.001
Age (years)				
< 40	45	694	4.8 (4.2) per	0.258
40–49	124	653	decade	
50–59	358	691		
60–69	772	718		
70–79	404	694		
≥ 80	55	689		
Smoking status				
Never smoker	634	696	−21.0 (6.8) per	0.002
Former smoker	795	717	pack of 20	
Current smoker (cigarettes/day)			cigarettes	
1–19	56	656		
20–39	167	670		
≥ 40	102	676		
Quetelet index (kg/m^2)				
< 21.0	174	644	3.5 (2.3) per	0.131
21.0–22.9	287	688	two units	
23.0–24.9	436	709		
25.0–26.9	377	724		
27.0–28.9	236	704		
≥ 29.0	210	700		
Current vitamin use				
None	998	692		
Occasional	273	693	7.9 (11.5)	0.495
Daily	420	731	45.7 (9.9)	<0.001

* Univariate analysis.

† Regression coefficient (standard error (SE)) adjusted for center, season, and all other personal characteristics.

‡ For test of significance of adjusted regression coefficients.

current cigarette smoking. Previous studies have shown differing results with respect to the association of beta-carotene with either age or Quetelet index. We found that Quetelet index, but not age, was negatively associated with plasma beta-carotene levels. Finally, we found that the use of beta blockers and other antihypertensive drugs was associated with clinically small but statistically significant decreases in beta-carotene levels. A previous study demonstrated that use of antihypertensive medications (mostly diuretics) was related to a decrease in beta-carotene levels (16). We found that current diuretic use was associated with decreased beta-carotene levels which narrowly failed to achieve statistical significance (adjusted proportional change = 0.93, 95 per cent CI 0.86–1.01).

As would be true for any drug not synthesized by the body, steady state plasma concentration of beta-carotene must be determined by oral dose, oral bioavailability, binding to plasma components, and clearance. Thus, the personal characteristics and drug classes that appear to have positive or negative effects upon plasma carotene levels must affect one or more of these pharmacokinetic variables. The outline of the pharmacokinetics of beta-carotene has been described, even though many details are lacking (31, 32). Of carotene ingested, only one third or less is absorbed as intact beta-carotene. Absorption depends on sev-

TABLE 4

Mean plasma retinol level (ng/ml) and adjusted regression coefficients according to medication use

	n	Mean*	Adjusted regression coefficient (SE)†	*p* value‡
Diuretics				
None	1,319	682		
Current use	428	758	73.8 (10.6)	<0.001
Beta blockers				
None	1,513	692		
Current	234	757	36.0 (12.7)	0.005
Other antihypertensives§				
None	1,618	697		
Current use	129	749	−8.4 (16.7)	0.615
Nitrates				
None	1,665	700		
Current	82	715	−48.9 (21.2)	0.021
Digoxin				
None	1,669	701		
Current	78	700	−24.2 (19.8)	0.222
Other cardiovascular drugs§				
None	1,693	698		
Current	54	782	75.5 (25.8)	0.004
Menopausal estrogens (women only)				
None	463	654		
Current use	70	703	57.6 (21.4)	0.007

* Univariate analysis.

† Regression coefficient (standard error (SE)) adjusted for center, season, personal characteristics, and all other drug use.

‡ For test of significance of adjusted regression coefficients.

§ See text for details.

eral factors including the presence of bile and fat, and the administration of other substances (e.g., mineral oil, dispersing agents). Enzymes in the intestinal wall can also cleave beta-carotene to form two molecules of retinal, which can be oxidized to retinoic acid or reduced to retinol. When beta-carotene is absorbed intact, it is transported in chylomicrons to the liver, and then secreted within the hydrophobic cores of very low density lipoprotein particles into the blood. Since very low density lipoprotein particles are converted in the plasma into low density lipoprotein particles, most beta-carotene in plasma appears to be bound within the core of low density lipoprotein particles. This helps explain the positive relation between beta-carotene levels and low density lipoprotein cholesterol (33).

It is clear that recent dietary intake of carotene is a major determinant of beta-carotene levels. The large interindividual variability in plasma levels, after reported intake has been considered, may in part reflect imprecision in the assessment of dietary carotene. There also may be differences in the oral bioavailabilities of the substance as encountered in the diet (33–37). For example, beta-carotene may vary in its bioavailability according to food preparation, and cooking, mashing, etc. may influence how efficiently it is absorbed. Our observation that a doubling of dietary beta-carotene intake is associated with only a 29 per cent increase in geometric mean levels is difficult to explain. For some vitamins and micronutrients such as iron (38), calcium (39), or retinol (32), increased dietary intake or increased total body stores lead to decreased bioavailability, but these absorptive processes are carrier-mediated and under physiologic control. There is no evidence that a similar system actually exists

which could reduce beta-carotene oral bioavailability as dietary intake increases.

It is not easy to explain why more obese persons have lower blood levels of beta-carotene, even after adjustment for dietary intake. It has been suggested that decreased plasma beta-carotene levels in heavier subjects may simply reflect a larger volume of distribution (larger fat stores) for this fat-soluble vitamin (10), but this explanation could be true only before a true steady state equilibrium has been reached. After that, the volume of distribution would not determine the steady state plasma beta-carotene level.

The association of current cigarette smoking with lower plasma levels of beta-carotene is also difficult to explain. In all studies which have investigated this issue, smokers have also had lower reported intake of dietary carotene, but this difference has not been large enough to account for the lower plasma levels. The findings suggest that in comparison with nonsmokers, smokers either over-report dietary carotene intake or that they have a change in beta-carotene pharmacokinetics, such as decreased bioavailability or increased clearance. Smoking alters metabolism in a number of ways, including raising the levels of glucose, cortisol, and adrenal androgens while decreasing the levels of leukocyte vitamin C, uric acid, albumin, and the high density lipoprotein cholesterol/low density lipoprotein cholesterol ratio (40, 41). Smoking can induce various hepatic enzymes, which may increase clearance (and reduce steady state plasma concentrations) of other drugs subject to hepatic biotransformation (40, 42). Interestingly, one study of 34 smokers and 34 matched controls found that while serum levels of beta-carotene and lutein were lower in smokers, levels of lycopene and retinol were similar (17); thus, the relation of smoking to carotenoid levels may differ for different compounds. In contrast to one earlier study (10), we did not find a significant interaction between cigarette smoking and dietary carotene intake in the regression of log plasma beta-carotene levels. The explanation for this difference in findings is not clear.

Our results for mean plasma retinol levels are similar to those previously reported (5, 10, 16, 30). We have confirmed that women have substantially lower retinol levels than men. Our data also indicate that several other factors may be independently associated with retinol levels. The use of vitamins, diuretics, estrogens, beta blockers, and other cardiovascular drugs was associated with higher plasma retinol levels. Interestingly, we also found that smoking cigarettes and use of nitrates were negatively related to retinol levels. It is difficult to explain these latter observations. Retinol in plasma is bound to retinol binding protein which is secreted by the liver, and retinol binding protein concentrations (and, hence, retinol levels) remain relatively stable throughout a wide range of dietary intake (32). On the other hand, changes in retinol binding protein concentrations lead to changes in retinol levels, as occurs with administration of estrogens (21, 32). However, this line of reasoning would not explain why men have higher retinol levels than women.

The effects of diuretics, beta blockers, other antihypertensive drugs, or other cardiovascular drugs on raising retinol levels are probably not due to the effects of ischemic heart disease, since the use of nitrates or digoxin did not produce the same effects. Diuretics raise low density lipoprotein cholesterol levels, and beta blockers can have a similar effect (43). The effects of medication use on retinol levels are not likely to be caused by drug-induced changes in lipoproteins or cholesterol levels, however, unless there also are associated changes in circulating levels of retinol binding protein/prealbumin as well (44). Medication use could also affect either absorption of retinol, or the ability of the intestinal mucosa to esterify retinol to form retinyl esters, which can then be transported within chylomicrons. These mechanisms have been hypothesized to explain lower vitamin A

status in patients with cystic fibrosis (34). At this time, however, there are no data concerning the effects of any of these classes of drugs upon retinol pharmacokinetics.

An important strength of our study is that the high-performance liquid chromatographic assays used to determine retinol and beta-carotene are sensitive, specific, precise, and accurate. Our results thus avoid the uncertainties of some earlier studies which analyzed only total carotenoids using spectrophotometric assays. Also, the size of our study allowed us to detect relatively weak relations between blood levels and personal factors or medication use. Weaknesses of the study are that the study group was not selected to be representative of the general population, but was chosen for participation in a clinical trial of cancer prevention; that subjects were not systematically questioned regarding medical history and alcohol intake at the time of randomization; and that we did not measure plasma concentrations of lipoproteins, and were therefore unable to control for these possible covariates.

Further clinical studies are needed to define the pharmacokinetic mechanisms for the associations between beta-carotene and retinol levels and the factors we identified as clinically important in this study. As our clinical trial progresses, we will obtain information on variables which determine the response of plasma beta-carotene or retinol to daily supplements of beta-carotene. For example, it will be important to learn whether smoking is related only to lower baseline beta-carotene levels or if it decreases the response to beta-carotene supplementation. These data may prove useful in the design and interpretation of both observational studies and intervention trials and may also help to explain the human pharmacology of retinol and beta-carotene.

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