Effect of Supplementation with β-Carotene and Vitamin A on Lung Nutrient Levels[1]

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Abstract

The Carotene and Retinol Efficacy Trial (CARET), a randomized, placebo-controlled lung cancer chemoprevention trial of 30 mg of β-carotene and 25,000 IU of retinyl palmitate, was prematurely terminated when a 46% excess lung cancer mortality was found in subjects on the active arm. Before the CARET intervention ended, 21 men were recruited to participate in a 6-month biomarker study using the same intervention as CARET that determined the effect of this supplementation on lung nutrient levels. Plasma and bronchoalveolar lavage (BAL) cell nutrient levels were measured before and after the intervention. The group in the active arm ($n = 10$) had plasma β-carotene level increases of over 10-fold, with a small increase in plasma retinol levels. BAL cell levels of β-carotene in the active group also increased 10-fold, from 4.5 to 46.3 pmol/10^6 cells ($P = 0.0008$), with no change in BAL cell retinol levels. Surgically obtained lung tissue from three CARET subjects in the active arm showed elevated β-carotene lung tissue levels but no increase in lung retinol levels compared to a group of surgical controls. Combined with our previous work showing a strong correlation between BAL and lung tissue nutrient levels, these findings suggest that supplementation with β-carotene and vitamin A results in increased lung tissue as well as BAL cell levels of β-carotene, with little change in lung retinol.

Introduction

A large number of epidemiological and experimental studies have suggested that β-carotene and retinol (vitamin A) may have cancer-preventive effects (1–3), prompting several large chemopreventive trials (4). CARET,[3] a multicenter randomized trial to assess the chemopreventive efficacy of the combination of 30 mg of β-carotene and 25,000 IU of retinyl palmitate on the risk of lung cancer, was terminated prematurely due to the findings of a 46% excess lung cancer mortality in the active intervention group (5). These findings were consistent with another lung cancer prevention trial, which also demonstrated an increased risk of lung cancer in participants receiving β-carotene (6).

Despite this increasing interest in the role of dietary factors in lung cancer and other diseases, surprisingly little is known about the storage and metabolism of these nutrients in human tissues, especially the lung, and even less is known on the effects of either short-term or chronic supplementation (7–9). We have recently demonstrated quantifiable levels of retinol, α-tocopherol, and total carotenoids or β-carotene in human lung tissue and BAL cells in a group of patients undergoing thoracic surgery. Lung tissue levels of total carotenoids, β-carotene, and α-tocopherol correlated well with plasma levels, whereas lung tissue levels of retinol correlated best with BAL cell levels (10). Thus, plasma and/or BAL nutrient levels may reflect lung tissue levels and may serve as more accessible biomarkers for the lung.

The current study was performed as part of a 6-month randomized, controlled study of the effects of the CARET intervention, β-carotene, and retinyl palmitate on markers of lung nutrient status and carcinogenesis (11). Subjects who met the same criteria but had not participated in CARET were recruited to participate. Data on the BAL and plasma nutrient levels on the 21 subjects are presented here. For comparison, we also present data on 12 CARET participants who had been on the active intervention who underwent bronchoscopy shortly after CARET was terminated and on 4 additional CARET subjects on whom lung tissue was obtained.

Materials and Methods

Study Population. Twenty-one subjects who met the same eligibility criteria as the CARET trial (heavy asbestos exposure, current or prior smoking, age between 45 and 70 years, absence of severe liver disease, and not on vitamin A supplementation) were recruited to participate in this study, which used the same intervention as CARET (11). None were participants in CARET.

Twelve additional subjects were recruited from among active participants in the CARET study as follows: the CARET study was terminated prematurely in January 1996, with a mean of 5 years of follow-up after randomization. Sixty-five CARET participants followed in the New Haven Center were recruited to participate in a postintervention study. Fifty-two of these 65 agreed to participate, 29 of whom were later determined to be on the active intervention; 12 volunteered for bronchoscopy. Four additional CARET subjects undergoing thoracic surgery (three who had been on the vitamin arm and one who had been on placebo) were also recruited to participate. All studies were approved by the Yale Committee on Human Investigation, and informed consent was obtained from all subjects.

Lung nutrient data from a previous published study of non-CARET participants is also included as a comparison group (10). This population consisted of 21 surgical patients who had undergone lung resection, 12 of whom underwent simultaneous bronchoscopy (10).

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[3] The abbreviations used are: CARET, Carotene and Retinol Efficacy Trial; BAL, bronchoalveolar lavage.

Study Design. This study was designed as a randomized, two-arm, placebo controlled, double-blinded trial (11). Baseline studies, including plasma nutrient levels were performed on each eligible subject. Questionnaires were administered to provide data on occupational exposures, cigarette smoking, and diet, as well as comparability to the CARET population. Subjects then underwent bronchoscopy and were randomly assigned to receive either placebo or 30 mg of β-carotene and 25,000 of retinyl palmitate. At 6 months, the full protocol, including bronchoscopy, was performed again on each subject. Subjects were contacted monthly to assess compliance and potential side effects. Bronchoscopy was performed on the 12 CARET subjects a mean of 18 days (range, 7–34) after stopping the vitamin intervention. Lung tissue samples were collected on the four CARET subjects undergoing thoracic surgery from areas of lung distant from the areas of suspected pathology. Approximately 1–2 g of lung tissue was obtained from each patient, protected from light, and promptly frozen at −70°C.

Fiber Optic Bronchoscopy and Processing of Samples. Fiber optic bronchoscopy and processing of blood and BAL samples was performed as described in prior publications (10, 12). All subjects were lavaged at identical sites to the extent possible. A minimum of 5 million BAL cells were required for the nutrient analyses. All samples were stored at −70°C prior to analysis and were protected from light during their processing.

Nutrient and Dietary Analyses. Plasma, BAL, and lung tissue levels of retinol, retinyl esters (retinyl palmitate, stearate, linoleate, and oleate), α-tocopherol, and the common dietary carotenoids (α-carotene, β-carotene, lycopene, zeaxanthin/lutein, and β-cryptoxanthin) were measured by reverse-phase high-performance liquid chromatography with minor modification of the method of detection of these compounds from the previously described procedures (10, 13, 14). The retinoids, α-tocopherol, and carotenoids were detected simultaneously using a Waters 996 Photodiodearray detector. Full spectra were collected for every fifth sample analyzed to confirm the validity of our peak identifications and to assess for interfering substances. Our lower limits of detection for retinol, carotenoids, and α-tocopherol were less than 0.01, 0.04, and 0.46 nmol/g, respectively, for tissue; 0.01, 0.02, and 0.23 μmol/liter, respectively, for plasma; and 1.3, 2.6, and 23.2 μmol, respectively, for 10^6 BAL cells. The total carotenoid level represents the sum of each of the individual carotenoid levels measured in the sample.

Daily dietary nutrient intake for each patient was assessed using the food frequency questionnaire and computer algorithms used by CARET as described previously (15, 16). Data are presented as daily dietary intake of retinol, β-carotene, total carotenoids, and α-tocopherol.

Data Analysis. Values are expressed as the mean ± SD. Student's *t* test was used to compare baseline characteristics and nutrient levels between comparison groups. A paired sign test was used to compare levels before and 6 months after the intervention on paired samples. The α level used to indicate statistically significant outcomes in two-tailed comparisons was 0.05. Data Desk 5.0 (Data Description Inc., Ithaca, NY) and SAS statistical programs were used for the analysis.

Results

Characteristics of the Participants. Twenty-one subjects were enrolled in the biomarker study. All of the subjects were male, with a mean age of 64 years; the majority were former smokers with a mean of 24.8 pack-years of smoking (Table 1). The demographic characteristics of the 21 participants in the biomarker study and the 12 CARET subjects were similar, with no significant differences in age, smoking status, asbestos exposure, or baseline dietary intake (Table 1 and data not shown). Daily dietary intake of β-carotene, retinol, and total vitamin A were similar in the groups and comparable to the larger CARET population (16). The biomarker study subjects were on the intervention for 6 months; the mean length of the intervention for the CARET subjects was 5 years. The four CARET patients who underwent thoracic surgery had a mean age of 67 years; three had been on the active CARET intervention for a mean of 5 years, and one had been on the placebo for 4 years.

Table 1 Baseline demographic information of biomarker participants[a]

	Placebo $n = 11$	Active $n = 10$
Age (yr)	62 ± 9	65 ± 6
Smoking		
No. current	3	2
No. former	6	6
No. never	2	2
Pack-yr	22 ± 17	28 ± 24
Asbestos exposure		
No. currently exposed	1	1
No. with past exposure	10	9
No. yr of exposure	32 ± 10	34 ± 11
Chest X-ray		
Normal	1	2
Plaques	9	6
Plaques/asbestosis	1	2
Dietary nutrients		
Retinol (μg/day)	505 ± 451	566 ± 275
β-Carotene (μg/day)	3574 ± 1755	2887 ± 2201
Vitamin A (μg RE/day)[b]	1098 ± 568	1003 ± 507

[a] $P > 0.05$ for comparison between intervention arms.
[b] RE, retinol equivalents.

Plasma Nutrient Levels. Carotenoids (α-carotene, β-carotene, lycopene, zeaxanthin/lutein, and β-cryptoxanthin), retinol, and α-tocopherol levels were determined for all samples. Retinyl esters were not detected in any of the plasma, BAL cell, or lung tissue samples; all retinol levels presented represent free retinol. Plasma nutrient levels pre- and postintervention are shown in Table 2. Baseline plasma β-carotene and retinol levels were comparable in the active and placebo groups (Table 2).

Six-month supplementation with β-carotene and retinyl palmitate resulted in significantly increased levels of plasma β-carotene, with minimal increase in plasma retinol levels (Table 2). These retinol levels are consistent with the known tight regulation of plasma retinol levels.

BAL Cell Nutrient Levels. BAL differential cell counts revealed over 85% macrophages, the remainder being, primarily, lymphocytes and neutrophils. BAL cell nutrient levels are shown in Table 3. In the biomarker study, β-carotene levels in the BAL cells were significantly increased (from 4.5 ± 4.8 to 46.3 ± 53.3 pmol/10^6 cells; $P = 0.0008$) following 6 months of supplementation compared to the placebo group, which showed no change in BAL cell β-carotene levels (Table 3). Somewhat lower but still significantly elevated BAL cell β-carotene levels were also found in the CARET participants (data not shown). Our prior findings that lung tissue β-carotene levels correlated best with plasma levels suggests that supplementation with β-carotene increased lung tissue β-carotene levels (10).

Interestingly, BAL cell retinol levels did not increase following 6 months of supplementation, as shown in Table 3. BAL cell retinol levels from the CARET participants who had been on more long-term supplementation were higher compared to levels in those on short-term supplementation (6.64 ± 5.94 *versus* 2.6 ± 2.6 pmol/10^6 cells). However, the BAL cell retinol levels were still quite low and were

Table 2 Plasma nutrient levels in biomarker study before and after intervention (μmol/liter)

	Preintervention	Postintervention
Retinol		
Placebo	1.53 ± 0.58	1.59 ± 0.47
Active	1.53 ± 0.37	1.87 ± 0.36
β-Carotene		
Placebo	0.31 ± 0.40	0.29 ± 0.31
Active	0.29 ± 0.20	3.67 ± 2.51[a,b]
Vitamin E		
Placebo	39.25 ± 14.88	31.57 ± 10.29
Active	38.83 ± 20.81	35.11 ± 10.92
Zeaxanthin/lutein		
Placebo	0.28 ± 0.17	0.20 ± 0.09
Active	0.18 ± 0.09	0.18 ± 0.09
Cryptoxanthin		
Placebo	0.12 ± 0.14	0.08 ± 0.07
Active	0.09 ± 0.06	0.15 ± 0.11
Lycopene		
Placebo	0.89 ± 0.41	0.76 ± 0.29[c]
Active	0.53 ± 0.35	0.60 ± 0.34
α-Carotene		
Placebo	0.07 ± 0.08	0.05 ± 0.08
Active	0.03 ± 0.02	0.12 ± 0.07[c]
Total carotenoids		
Placebo	1.80 ± 1.04	1.38 ± 0.68
Active	1.12 ± 0.53	4.71 ± 2.40[a,b]

[a] $P < 0.01$ for comparison between active and placebo groups.
[b] $P < 0.01$ for paired test from preintervention to postintervention.
[c] $P < 0.05$ for paired test from preintervention to postintervention.

Table 3 BAL cell nutrient levels in biomarker study before and after intervention (pmol/10^6 cells)

	Preintervention	Postintervention
Retinol		
Placebo	1.3 ± 1.9	4.1 ± 3.9
Active	1.8 ± 1.8	2.6 ± 2.6
β-Carotene		
Placebo	3.2 ± 4.3	3.6 ± 6.8
Active	4.5 ± 4.8	46.3 ± 53.3[a,b]
Vitamin E		
Placebo	358.7 ± 478.1	265.7 ± 255.9
Active	159.0 ± 92.8	263.5 ± 257.1
Zeaxanthin/lutein		
Placebo	6.2 ± 5.0	5.7 ± 6.0
Active	6.9 ± 6.4	4.4 ± 2.3
Cryptoxanthin		
Placebo	1.2 ± 1.1	2.1 ± 3.2
Active	3.5 ± 3.9	3.4 ± 2.9
Lycopene		
Placebo	8.2 ± 9.7	3.5 ± 2.8
Active	7.4 ± 5.4	4.3 ± 3.3
α-Carotene		
Placebo	0.6 ± 0.5	1.0 ± 1.3
Active	0.5 ± 0.5	1.9 ± 1.2[c,d]
Total carotenoids		
Placebo	17.1 ± 12.8	16.1 ± 16.2
Active	22.4 ± 19.8	60.3 ± 58.0[c]

[a] $P < 0.01$ for comparison between active and placebo groups.
[b] $P < 0.01$ for paired test from preintervention to postintervention.
[c] $P < 0.05$ for comparison between active and placebo groups.
[d] $P < 0.05$ for paired test from preintervention to postintervention.

comparable to our prior study of non-CARET surgical patients (6.12 pmol/10^6 cells; Ref. 10). Our prior findings demonstrating that BAL cell retinol levels but not plasma levels correlated with lung tissue retinol levels suggests that retinol and β-carotene supplementation for 6 months did not result in an increase in lung retinol levels.

Interactions with Other Nutrients. We found no difference in plasma vitamin E, zeaxanthin/lutein, or β-cryptoxanthin levels between the placebo and active groups. The increase in plasma α-carotene found in subjects on active intervention may reflect some contamination of the pills with α-carotene. Similarly, no such differences in other nutrients were seen in the BAL samples, except for a similar increase in α-carotene in participants in the active vitamin arm.

Lung Tissue Levels. Fresh lung tissue was fortuitously available from four CARET participants: two on active intervention, one on placebo, and one active participant who had been off the intervention for 5 months. Carotenoid, retinol, and α-tocopherol levels on these samples are shown in Table 4. For comparison, also shown are nutrient levels that we had previously obtained on a similar group of 21 surgical patients not in the CARET study (10). The β-carotene lung tissue levels in the two subjects who were on the active intervention are increased compared to the comparison group. Of interest, lung tissue retinol levels in the two subjects on active intervention for a mean of 5 years remained low and contained no retinyl esters, similar to the surgical patients. These retinol lung tissue data are consistent with the BAL retinol data that showed little increase in BAL retinol levels, even with long-term supplementation. Nutrient levels in the one CARET participant who had been off the intervention for 5 months was comparable to the placebo patient and comparison group (or surgical patients).

Table 4 Lung nutrient levels (nmol/g)

	Active[a] $n = 2$ (mean)	Placebo[a] $n = 1$	Active[a] $n = 1$ (off 5 months)	Controls[b] $n = 21$ (mean ± SD)
Retinol	0.56	0.74	0.33	0.52 ± 0.21
β-Carotene	0.76	0.31	0.16	0.24 ± 0.50
Vitamin E	13.30	29.21	6.27	22.29 ± 11.38
Zeaxanthin/lutein	0.13	0.31	0.13	0.12 ± 0.09
Cryptoxanthin	0.82	0.13	0.06	0.07 ± 0.07
Lycopene	0.15	0.51	0.16	0.17 ± 0.11
α-Carotene	0.04	0.06	0.03	0.06 ± 0.04
Total carotenoids	1.89	1.33	0.53	0.66 ± 0.81

[a] CARET participants who underwent thoracic surgery while still on the CARET intervention or 5 months after stopping the intervention.
[b] Nutrient levels obtained in 21 non-CARET participants undergoing thoracic surgery or open lung biopsy.

Discussion

Much attention has been focused on the role of β-carotene and vitamin A in carcinogenesis and cancer prevention and the potential benefits of supplementation with such dietary agents (1). However, little is known about human lung storage and metabolism of these nutrients, and even less is known about the consequences of supplementation, in large part due to the inaccessibility of human lung tissue (8, 9). The purpose of this study was to determine the effect of both short-term (6 months) and more long-term (mean, 5 years) supplementation with β-carotene and retinyl palmitate on lung nutrient levels. As shown here, the 6-month intervention resulted in markedly increased BAL cell β-carotene levels but minimal increase in lung BAL retinol levels. More long-term supplementation in CARET subjects resulted in similarly increased BAL cell

β-carotene levels and slightly increased but still low retinol levels. These findings can be interpreted in the context of our recent findings, which demonstrated that plasma β-carotene and BAL retinol levels best predicted lung tissue β-carotene and retinol levels, respectively, and may serve as more accessible markers to assess lung nutrient status. The data presented here thus suggest that combined β-carotene and retinol supplementation in humans can significantly increase lung β-carotene levels but has little effect on lung retinol levels.

The effects of oral supplementation with β-carotene and/or retinol on lung nutrient levels in animals have received little attention; most studies have focused on the liver. The effects of oral supplementation on tissue nutrient levels have been quite variable depending on the organ, species, other dietary nutrients, and administration protocol, making it difficult to predict the effects of such supplementation in humans based on animal studies. Certain species, such as pigs, are able to take up and accumulate β-carotene in the lung following supplementation, whereas other species, such as rats, do so to a much lesser degree (17–19). In different species, vitamin A-supplemented diets have been shown to result in quite variable lung tissue retinol levels (7, 17, 20). Human data on the effects of dietary supplementation on tissue nutrient stores are very limited.

As far as we are aware, this study is the first to document the effect of supplementation with vitamin A and β-carotene on human lung BAL cell and lung tissue retinol and β-carotene levels. One recent study reported a 4-fold increase in BAL β-carotene cell levels in four subjects following 6 weeks of supplementation with combined vitamin E, vitamin C, and β-carotene (22.5 mg/day; Ref. 21). Our findings of an 8–12-fold increase in BAL β-carotene may reflect the longer duration and higher dose of our intervention. We are aware of no prior studies investigating the effects of vitamin A supplementation on human lung BAL or tissue levels.

Interactions between different carotenoids, retinoids, and other nutrients both in plasma and, importantly, at target end organs, such as the lung, are poorly understood. β-Carotene is one of several hundred carotenoids present in the diet; a number of different retinoids, and retinoid isomers and metabolites also exist. Important interactions between these multiple different nutrients may occur (8, 20). Whether supplementation with vitamin A and β-carotene can alter the uptake, storage, or metabolism of any other nutrients is an important question and warrants further investigation. Recent studies have found conflicting effects of β-carotene supplementation on other serum nutrient levels (22, 23). We found no difference in both plasma and BAL cell vitamin E, zeaxanthin/lutein, or β-cryptoxanthin levels between the placebo and active groups. The increase in α-carotene found in subjects on active intervention may reflect some contamination of the pills with α-carotene or a real effect. We are aware of no data addressing this question of nutrient interactions at the lung tissue level, and unfortunately, our small sample size makes it difficult to address this question.

The major limitation of this study is the small sample size, especially of the lung tissue samples. The number of subjects was limited by several factors, primarily the unexpected cessation of the CARET intervention, and also by the time and costs involved in performing bronchoscopy. Recruitment to the biomarker study was halted at the same time for ethical reasons, so it was not possible to increase the number of subjects in this study. The lung tissue analysis was limited because the nutrient assays require fresh or frozen lung tissue and the CARET study was not designed to facilitate the collection of such tissue. Unfortunately, we were unable to measure β-carotene metabolites in the BAL cells.

In conclusion, this study found that the CARET intervention resulted in increased lung BAL cell and lung tissue β-carotene levels but little or no increase in lung BAL cell and lung tissue retinol levels. Further studies on the effects of supplementation and potential interactions between different carotenoids, retinoids, and other nutrients are warranted.

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