Effect of β-Carotene Supplementation on the Risk of Pneumonia Is Heterogeneous in Males: Effect Modification by Cigarette Smoking

Harri HEMILÄ

Department of Public Health, University of Helsinki, POB 41, Helsinki, FI-00014, Finland

(Received February 28, 2018)

Summary Beta-carotene has been suggested to be a factor for improving the immune system, which implies that it might decrease the risk of infections. We therefore analyzed whether beta-carotene supplementation influenced pneumonia risk in 14,564 Finnish male smokers of the Alpha-Tocopherol Beta-Carotene (ATBC) Study. There were 231 pneumonia cases in the beta-carotene group and 217 cases in the placebo group. Thus, beta-carotene had no effect on the average incidence of pneumonia, RR=1.07 (95% CI: 0.89–1.29). However, cigarette smoking exposure significantly modified the effect. Beta-carotene increased pneumonia risk by RR=4.0 (95% CI: 1.63–10) among 990 participants who started to smoke at the age of ≥21 y and smoked ≥21 cigarettes per day at the study baseline. However, beta-carotene had no influence on pneumonia risk for the remaining participants. We also analyzed the effect of beta-carotene on participants who quit smoking during the ATBC Study. Among 4,290 participants who quit smoking, the 58 pneumonia cases were evenly distributed between the beta-carotene and placebo groups with RR=0.93 (95% CI: 0.55–1.55). Accordingly, no evidence was found that beta-carotene decreased pneumonia risk; instead, it significantly increased the incidence of pneumonia in a subgroup that covered 7% of the study population.

Key Words dietary supplements, epigenetics, immune system, randomized controlled trial, respiratory tract infections

Studies on laboratory measures of the immune system led to the proposal that β-carotene might enhance immune functions (1,2). In addition, a negative correlation between plasma β-carotene level and the incidence of acute respiratory infections in elderly people was reported, which indicated that higher intake levels of β-carotene might protect against respiratory tract infections (3). However, the large-scale Alpha-Tocopherol Beta-Carotene Study on Finnish male smokers found that β-carotene supplementation did not influence the average incidence of common colds (4) or the average incidence of pneumonia (5). Nevertheless, the effect of β-carotene on common cold incidence was heterogeneous; it was modified by the age of the participant when he had initiated smoking and by the level of smoking at the baseline of the study (4). The effect of β-carotene on the incidence of pneumonia was modified by the age of the participant when he had initiated smoking (5). The purpose of this analysis was to explore whether the age of smoking initiation and the level of smoking at the baseline might together influence the effect of β-carotene supplementation on the risk of pneumonia in the ATBC Study cohort.

Participants and Methods

The design and methods of the ATBC Study, which examined the effects of α-tocopherol (AT) and β-carotene (BC; 20 mg/d) on the incidence of lung cancer and other cancers have been described in detail (5–7). The ATBC Study is registered at ClinicalTrials.gov under the identifier NCT00342992. In brief, male participants aged 50–69 y were eligible for inclusion if they smoked ≥5 cigarettes/d at entry and those enrolled in the trial (n=29,133) were randomized into one of four intervention arms, and were administered a placebo, AT, BC, or AT+BC, using a 2×2 factorial design. The intervention continued for 5 to 8 y until April 1993. The trial was approved by the institutional review boards and all participants gave their written informed consent. Since statistically significant interaction between the effects of AT and BC was previously observed in certain subgroups of the ATBC Study (8,9), the current analysis was restricted to the no-AT participants; thus, the comparison in this study was between the BC and the placebo arms. In addition, the age at smoking initiation was missing from 5 participants and they were excluded. Thus, the data set of this analysis included 14,564 participants.

Before randomization, the men completed questionnaires on their medical and smoking histories. There were 3 follow-up visits annually (i.e., at 4-mo intervals). At each follow-up visit, the subject was asked, “Have you been smoking since the previous visit?” with the following alternative responses provided: (1) no, (2) yes, but now I have quit, and (3) yes, continuously.

The outcome of this analysis was the first hospital-treated pneumonia after randomization (5). Because
almost all of the participants lived at home, the pneumonia cases represent primarily community-acquired pneumonia. The follow-up time for each participant began from the day of randomization, and continued until the occurrence of pneumonia, death, or the end of the trial, whichever event occurred first. There were 448 pneumonia cases during the 84,049 person-years of observation. The median follow-up time of the present analysis was 6.0 y.

The effect of β-carotene on the incidence of pneumonia was estimated by Cox regression models using the coxph-procedure of the R-package (https://www.r-project.org). The statistical significance of the improvement in the model was calculated from the change in $\chi^2$ log (likelihood) when the additional terms were added to the Cox model. The risk ratio of pneumonia was calculated with the riskratio procedure of the fmsb package of the R. See the Supplemental Online Material for the statistical calculations.

Results
The β-carotene supplementation had no effect on the average incidence of pneumonia in the 14,564 participants, with RR = 1.07 (95% CI: 0.89–1.29). There were 231 cases of pneumonia in the β-carotene group and 217 cases in the placebo group. However, there was significant heterogeneity in the effect of β-carotene by the level of cigarette smoking exposure (Table 1). The β-carotene supplementation had no effect on the inci-
The incidence of pneumonia substantially increases with age and the difference between the placebo and the \( \beta \)-carotene groups was most prominent at the upper age range (Fig. 1B). The difference between the Kaplan-Meier curves in Fig. 1B indicates the proportion of participants supplemented with \( \beta \)-carotene who contracted pneumonia because of \( \beta \)-carotene. The cumulative incidence of pneumonia by the age of 72 y was 1.9% in the placebo group and 12.7% in the \( \beta \)-carotene group, which indicates that 10.8% of participants in the \( \beta \)-carotene group contracted pneumonia because of the \( \beta \)-carotene administration.

All participants of the ATBC study had smoked at the start of the trial. However, during the follow-up 4,290 of the 14,564 participants reported that they had quit smoking on one or more visits. If \( \beta \)-carotene was capable of preventing pneumonia in males who do not currently smoke, a difference between the \( \beta \)-carotene and placebo groups would be expected to emerge after participants quit smoking. Consequently, three types of participant selections were used to estimate the occurrence of pneumonia after they had quit smoking. First, 58 pneumonia cases that occurred after any number of visits when the participant reported not to smoke were analyzed. Second, pneumonia cases that occurred after at least 1 y of not smoking were analyzed, and third, cases that occurred after at least 2 y of not smoking were analyzed. The incidence of pneumonia cases that occurred after the participants quit smoking did not differ between the \( \beta \)-carotene and placebo groups in any of the three selections (Table 2). See Figure S1 in Supplemental Online Material for the description of the 58 cases of pneumonia that occurred after quitting to smoke.

### Discussion

Contrary to the suggestions that \( \beta \)-carotene might improve immune functions and protect against respiratory infections (1–3), this analysis found that \( \beta \)-carotene administration led to a 4-fold increase in the risk of pneumonia for one subgroup of the ATBC Study, namely heavy smokers who had initiated smoking late in their lives. In this subgroup, 11% of the participants who were administered \( \beta \)-carotene contracted pneumonia by the age of 72 y because of the \( \beta \)-carotene administration. Previous analyses of the ATBC Study found that \( \beta \)-carotene supplementation did not prevent the common cold, but increased its incidence in certain subgroups (4, 8). \( \beta \)-Carotene had no overall effect on the risk of pneumonia (5), and neither did it influence the risk of pneumonia in participants who were under heavy physical stress, which causes oxidative stress (10). In addition, \( \beta \)-carotene did not prevent tuberculosis (11). Thus, the seemingly positive effects of \( \beta \)-carotene on certain laboratory-based measures of the immune system do not seem to manifest as clinically relevant effects against infections in this population of Finnish male smokers.

In the current study, the harm of \( \beta \)-carotene, revealed as the increase in pneumonia risk, was limited to 7% of the ATBC Study population. It is worth noting that there was no harm, but also no benefit, to the great majority covering 93% of the study population. However, even though the substantial harm of \( \beta \)-carotene supplementation was restricted to a limited proportion of heavy male smokers, this effect may have substantial public health implications.
health importance since smoking is very common in males, for example, in several Asian countries (12, 13). On the other hand, the prevalence of tobacco smoking in Europe and the USA has substantially decreased (1, 3). The global markets of \( \beta \)-carotene, which include sales of it as supplements, is a multi-million-dollar business and \( \beta \)-carotene is available as single nutrient supplements and it is included in various multivitamin supplement combinations (14, 15).

One of the variables that defined the subgroup in which \( \beta \)-carotene increased the risk of pneumonia was smoking heavily at the start of the ATBC Study (Table 1). The specific biochemical mechanisms whereby \( \beta \)-carotene causes harm to heavy smokers are not well known, though there is evidence which indicates that current smoking substantially modifies \( \beta \)-carotene metabolism. Plasma \( \beta \)-carotene levels are decreased in current smokers (16–18), whereas smoking cessation leads to an increase in \( \beta \)-carotene concentrations (19). Cigarette smoke exposure of plasma or \( \beta \)-carotene containing solution led to the degradation of \( \beta \)-carotene into numerous cleavage products (20–22). Cigarette smoke exposure in bronchial epithelial cells also led to the generation of several cleavage products of \( \beta \)-carotene (23).

Some of the \( \beta \)-carotene cleavage products may have toxic effects. They have been reported to inhibit \( \text{Na}^+\text{K}^+\text{ATPase} \) activity (24), impair mitochondrial respiration (25), and modify the respiratory burst of neutrophils (26). The combination of \( \beta \)-carotene and cigarette tar in lung microsomal membranes caused greater lipid peroxidation than either of the two substances alone (27), which might explain why \( \beta \)-carotene appears harmful for heavy smokers in particular.

Ferrets have been used as an animal model to investigate the interaction between cigarette smoke exposure and \( \beta \)-carotene metabolism. Cigarette-smoke exposure decreased \( \beta \)-carotene and retinoic acid levels in lung tissues, and cigarette smoke exposure combined with \( \beta \)-carotene administration caused histopathological changes in the lungs (28, 29).

The second variable defining the subgroup in which \( \beta \)-carotene administration increased the risk of pneumonia was the age of smoking initiation (Table 1). The statistical evidence in the current analysis that indicates different effects of \( \beta \)-carotene on participants who initiated smoking at \( \leq 20 \) vs. \( \geq 21 \) y is strong, yet the number of pneumonia cases is so small that this cut-off limit should not be interpreted as a biologically accurate estimate. Rather, a more robust characterization of the two groups may be made by considering their median ages, i.e., 18 vs. 24 y. Cigarette smoking causes dozens of epigenetic changes (30–32). Some of the DNA methylation changes have been shown to change gene expression. It is plausible that exposure to cigarette smoking at a younger age influences the developmental processes of the lungs and may lead to permanent changes, whereas initiating smoking at a later age might not cause similar changes. Thus, the different stages in lung development in those who started to smoke at 18 y and in those who started to smoke at 24 y might explain the differences in \( \beta \)-carotene effects found decades later (Table 1).

\( \beta \)-Carotene is not the only dietary supplement for which there is strong evidence of heterogeneity in the effects on the pneumonia risk. Previously, the effects of vitamin E supplementation on pneumonia risk in the ATBC Study was shown to be heterogeneous. Some groups of participants contracted pneumonia because of vitamin E administration (9, 33, 34). Nevertheless, vitamin E was beneficial by protecting against pneumonia for certain subgroups of the ATBC participants, namely for those who smoked least and exercised, and for those who quit smoking (9, 10, 34, 35). In contrast, no subgroups in the current study could be identified in which \( \beta \)-carotene supplementation would seem to be beneficial for the prevention of pneumonia (Tables 1 and 2).

In the 1980s, a few large-scale trials of \( \beta \)-carotene supplementation were initiated to determine whether it might decrease the incidence of lung cancer. Contrary to expectations, \( \beta \)-carotene increased the risk of lung cancer and overall mortality in smokers and asbestos-exposed participants (6, 36, 37). The current study indicates that \( \beta \)-carotene does not improve the immune system function in male smokers in such a way as to decrease the incidence of pneumonia even in those who quit smoking. Consequently, \( \beta \)-carotene self-supplementation should be discouraged, particularly for heavy smokers.

Acknowledgments

I thank the ATBC Study (The National Institute for Health and Welfare, Finland, and National Cancer Institute, USA) for the access to these data. I had full access to all of the data in this study and I take responsibility for the accuracy of the data analysis.

Supporting information

Supplemental Online Material is available on J-STAGE.

REFERENCES


