Short- and long-term β-carotene supplementation do not influence T cell–mediated immunity in healthy elderly persons1–4

Michelle S Santos, Lynette S Leka, Judy D Ribaya-Mercado, Robert M Russell, Mohsen Meydani, Charles H Hennekens, J Michael Gaziano, and Simin Nikbin Meydani

ABSTRACT Supplementation of healthy elderly persons with β-carotene has been considered a way to enhance immune responses. In study 1 the short-term effect of β-carotene (90 mg/d for 3 wk) on immunity was assessed in a randomized, double-blind, placebo-controlled longitudinal comparison of healthy elderly women. In study 2 the long-term effect of β-carotene (50 mg every other day for 10–12 y) on immunity was assessed in a randomized, double-blind, placebo-controlled longitudinal comparison of men enrolled in the Physicians’ Health Study. Subjects from both studies taking active supplements had significantly greater plasma β-carotene concentrations than did subjects taking placebo. The pre- to postintervention change in delayed-type hypersensitivity skin test responses between β-carotene and placebo groups in the short-term study was not significantly different, nor was the response between treatment groups in the long-term study. There were no significant effects due to β-carotene supplementation on in vitro lymphocyte proliferation, production of interleukin 2, or production of prostaglandin E2 as a result of short- or long-term β-carotene supplementation. In addition, there were no differences in the profiles of lymphocyte subsets (total T cells [CD3+], T helper cells [CD4+], T cytotoxic/suppressor cells [CD8+], and B cells [CD19+]) due to short- or long-term β-carotene supplementation; nor were there differences in percentages of CD16+ natural killer cells or activated lymphocytes (cells expressing interleukin 2 transferrin receptor) due to long-term β-carotene supplementation. Consistent results from these two trials show that β-carotene supplementation did not have an enhancing or suppressing effect on T cell–mediated immunity of healthy elderly. Am J Clin Nutr 1997;66:917–24.

KEY WORDS β-Carotene, immune response, elderly, men, women, T cell, delayed-type hypersensitivity skin test

INTRODUCTION

Immune function has been shown to decline in the elderly, with T cell–mediated immune function accounting for a large part of the decline (1). Incidence of infections (2) and diseases such as cancer, atherosclerosis, autoimmunity, and amyloidosis (3, 4) are higher in the elderly. Supplementation with specific antioxidant nutrients such as vitamin E has been shown to be effective in enhancing T cell–mediated immune responses in the elderly (5). Other antioxidants such as β-carotene may also be beneficial for reducing or delaying age-associated decreases in T cell–mediated immune function and subsequent onset of disease.

The effect of β-carotene supplementation on human lymphocyte subsets and expression of cell activation markers has been studied in some diseased populations (6, 7) as well as in young subjects (8). However, few human studies have investigated the effects of β-carotene supplementation on the function of immune cells in healthy young (9) or elderly individuals. Watson et al (10) conducted a variable-dose β-carotene supplementation trial (0, 15, 30, 45, and 60 mg/d) in a mixed-sex population (mean age: 56 y) with few subjects in each treatment group. They reported significant increases in the percentage of T helper cells and cells expressing the interleukin 2 receptor (IL-2R) and transferrin receptor (TIR) with β-carotene supplementation of 30–60 mg/d; however, no functional assessments of the immune cells were conducted.

Here we report the results of two distinct studies: a short-term, high-dose β-carotene supplementation trial in women

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3 The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.
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and a long-term, lower-dose β-carotene supplementation trial in men. Although different in design, both studies tested the hypothesis that β-carotene supplementation may enhance T cell–mediated immunity in elderly individuals.

SUBJECTS AND METHODS

Study design

Study design, subject characteristics, doses administered, and duration of the two studies are shown in Table 1 and summarized below.

Study 1: short-term effect of β-carotene on immunity of healthy elderly women

Healthy elderly females were recruited from the Boston area. Volunteers underwent a screening procedure that included a medical history, physical examination, routine urinalysis, and blood clinical chemistry profile. Volunteers without malabsorption problems, liver disease, diabetes, or elevated triacylglycerol or cholesterol concentrations (>10% of the upper limit of normal); who did not use steroids or other immunosensitive drugs; and who had not used vitamin supplements within the past 30 d were enrolled in the study. Subjects were nonsmokers and ingested alcohol occasionally. They were asked to refrain from alcohol use for ≥1 mo before beginning the study and during the study. Additionally, subjects did not take aspirin or nonsteroidal antiinflammatory drugs on a regular basis, nor <72 h before blood was drawn. All procedures were approved by the Human Investigation Review Committee of Tufts University Health Sciences. Informed, written consent was obtained from all participants.

Twenty-five healthy elderly women (aged 60–80 y, mean: 70 y) were randomly assigned to receive either 90 mg β-carotene/d (beadlets in 3 capsules of 30 mg each; Hoffmann-LaRoche, Nutley, NJ) or placebo (Hoffmann-LaRoche) for 23 d in this double-blind study. Subjects followed their normal diets and were instructed to take the β-carotene or placebo capsules with a high-fat breakfast (margarine or butter with bread). Delayed-type hypersensitivity (DTH) skin tests were administered on days 1 and 22 for analysis of in vivo immune function. Blood was drawn from fasting subjects on days 3, 4, 23, and 24 for analysis of in vitro immune function. Data generated from identical in vitro immune samples collected on days 3 and 4 were averaged to reflect baseline responses and data from identical samples collected on days 23 and 24 were averaged to reflect immune responses at follow-up. Statistical analyses and data presentation for in vitro immune responses are based on these averaged baseline and follow-up values. One subject did not complete the study because she could not give a volume of blood sufficient for investigation of immune indexes as a result of poor venous return. In addition, one subject was excluded because of a urinary tract infection, giving a final count of 11 subjects in the placebo group and 12 in the β-carotene group.

Study 2: long-term effect of β-carotene on immunity of men enrolled in the Physicians’ Health Study

The Physicians’ Health Study is a randomized, double-blind, placebo-controlled trial among 22,071 US male physicians testing low doses of aspirin and β-carotene (as 50 mg Luotonin on alternate days; BASF, Mount Olive, NJ) in the primary prevention of cardiovascular disease and cancer. With use of a 2 × 2 factorial design, subjects were randomly assigned to receive aspirin, β-carotene, both, or neither between 1982 and 1984. The randomized aspirin component was terminated in December, 1987, by the external Data Monitoring Board primarily as a result of a highly significant 44% reduction in risk of a first myocardial infarction in the aspirin group (11). The randomized β-carotene component continued uninterrupted until it ended as scheduled on December 31, 1995, representing an average of >12 y of treatment and follow-up (11).

Because of the need to obtain a fresh blood specimen for immunologic tests, a random sample of Boston area participants in the Physicians’ Health Study was recruited for this immunologic substudy. Single samples were collected between the months of July and November of 1993. This project was approved by the Internal Review Board of Brigham and Women’s Hospital and Harvard Medical School, and that approval was acknowledged by the Human Investigation Review Committee of Tufts University Health Sciences and New England

<table>
<thead>
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<th>TABLE 1</th>
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<td>Comparison of β-carotene supplementation studies</td>
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<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
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<tr>
<td><strong>Study type</strong></td>
<td>Longitudinal</td>
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<td>Duration</td>
<td>3 wk</td>
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</table>
| Subjects | Females aged 60–80 y (x = 70 y); placebo, n = 12. β-carotene, n = 11 | Males aged 50–86 y (x = 63 y)
placebo, n = 27. β-carotene, n = 27 |
| Dosage | 90 mg β-carotene/d | 50 mg β-carotene/2 d |
| Plasma β-carotene (μmol/L) | | |
| Baseline | | |
| Placebo | 0.68 ± 0.08 | NA² |
| β-Carotene | 0.74 ± 0.13 | NA² |
| Postintervention | | |
| Placebo | 0.69 ± 0.06 | 0.54 ± 0.06 |
| β-Carotene | 7.81 ± 1.93⁴,⁵ | 1.74 ± 0.1⁴,⁵ |

¹ Age at follow-up.
² Not available.
³ Significantly greater than baseline, P < 0.005 (Wilcoxon’s rank test).
⁴,⁵ Significantly greater than placebo: (Kruskal-Wallis nonparametric test) ⁴ P < 0.005, ⁵ P < 0.0001.
Medical Center hospitals. Informed, written consent was obtained from all participating physicians.

Recruitment was done by telephone. Investigators were blinded to β-carotene assignment. Subjects were asked to refrain from taking aspirin or other nonsteroidal antiinflammatory drugs for 10 d before blood was drawn. Subjects known to be taking extraneous vitamin supplements were excluded during recruitment to avoid confounding results. Blood samples from fasted subjects in both treatment and age groups were collected at the physicians’ residence or place of employment between 0630 and 1030; samples were collected from each group randomly across the given time period. Of 73 Boston area physicians participating, 19 were excluded from data analysis: 6 on the basis of use of aspirin within 72 h of the blood draw, 7 on the basis of use of supplements not provided by the Physicians’ Health Study, 5 on the basis of nonfasting, and 1 on the basis of low plasma β-carotene concentrations in the supplemented group.

Because of the nature of the study, physicians were admitted based on their willingness to collaborate as a part of the Physicians’ Health Study cohort. The population was considered healthy with no reported history of myocardial infarction, stroke, angina pectoris, cancer, or other major illnesses. In addition, 56% took no regular medications, 37% took 1–2 medications, and only 7% took 3 or more medications, the majority being antihypertensive and cholesterol-lowering drugs. As assessed by on-site questionnaire at the time of the blood draw, the physicians were in general good health. Compliance with supplement intake for 2 mo before blood collection was self-reported as a part of the questionnaire. 87% of the subjects reported taking all their pills and 8.6% missed 1–3 pills.

Plasma β-carotene analysis and skin test response

Plasma β-carotene concentrations were measured through use of reversed-phase gradient HPLC procedures as described previously (12, 13). Multitest CMI (Connaught Laboratories, Inc, Swiftwater, PA), a skin plant device with seven common antigens (tetanus, diphtheria, streptococcus, tuberculin, candida, trichophytion, and proteins) plus a glycerin control, was administered on the forearm by a nurse who was blinded to the study treatment assignment. Induration > 2 mm was considered to be a positive response and was measured at 48 h by the same investigator in a blinded manner. The total number of positive responses was recorded and the sum of the averages of individual inductions (mm) was recorded as the induration index.

Mononuclear cell isolation

All subjects fasted for 12 h before blood was drawn. Thirty milliliters blood was drawn into evacuated tubes with sodium heparin. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood with use of Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) density-gradient centrifugation. The mononuclear cell buffy layer was isolated and washed three times in complete media: RPMI 1640 (Sigma, St Louis) with 100 × 10^5 U penicillin/L and 100 mg streptomycin/L (Gibco BRL, New York), 2 mm glutamine/L (Gibco BRL), and 25 mmol HEPES/L (Sigma). Trypan blue staining was used to assess cell viability and lymphocytes were counted in a hemocytometer under a light microscope.

Lymphocyte proliferation

Lymphocyte proliferation cultures were prepared in triplicate (96-well, flat-bottomed plates; Falcon, Becton Dickinson and Co, Lincoln Park, NJ) with 1 × 10^6 cells/L in 10% autologous plasma. Cells were stimulated with phytohemagglutinin (PHA; Difco, Detroit) or concanavalin A (ConA; Sigma) at concentrations of 0.5, 5, and 50 mg/mL. Cultures were incubated in 5% CO2 at 37 °C. Cells were harvested onto glass filter paper at 72 h by using a cell harvester (PHD, Cambridge, MA) after a 4-h titrated thymidine pulse (18.5 kBq in 20 μL complete media added to 1 × 10^6 cells/well). Incorporation of [3H]thymidine into newly synthesized DNA was counted in a β scintillation counter (Beckman Instruments, Palo Alto, CA) and corrected counts per minute were converted to Bq.

Interleukin 2 and prostaglandin E_2 production

IL-2 and prostaglandin E_2 (PGE_2) cultures were prepared by plating 1.0 × 10^6 cells/L (24-well, flat-bottomed plates; Falcon) in 10% autologous plasma. PBMCs were stimulated by PHA and ConA (10 mg/L) for 48 h in 5% CO2 at 37 °C, after which supernates were harvested and frozen at -70 °C until analyzed.

A standard bioassay using the human IL-2–sensitive CTLL-2 murine cytotoxic cell line (developed in our lab from CTLL; ATCC, Rockville, MD) was used to determine the amount of IL-2 produced by the 48-h cultures; units of IL-2 activity were calculated by computer with probit analysis (14). This method was described in detail previously (5).

PGE_2 production was measured by radioimmunoassay as described previously (5). PGE_2 antibody (polyclonal rabbit γ-globulin) was kindly provided by J Dupont (Iowa State University, Ames) and M Mathias (Colorado State University, Fort Collins).

Lymphocyte subsets

Cell surface IL-2R and TIR expression were determined after culturing PBMCs (1 × 10^6 cells/L) in 10% autologous plasma (5 mL in 25-cm² Falcon tissue culture flasks). Cultures were stimulated with 5 mg PHA/L, the concentration that resulted in optimal expression of IL-2R and TIR at 48 h. Using a modified method of Durez et al (15), we removed surface-bound IL-2 and transferrin from IL-2R in preparation for tagging with monoclonal antibodies. Cells were washed with RPMI containing 20 mmol HEPES/L, pH 7.2, followed by a 2-min acid wash (25 mmol sodium acetate/L, pH 3.0; 100 μL per 3 × 10^6 cells). Cell suspensions were neutralized with RPMI, pH 9.0 (2.5 volumes per 1 volume acid medium), and an equal volume of RPMI containing 10% fetal bovine serum (pH 7.2). This procedure was carried out on ice to avoid transient internalization of IL-2R and TIR. Cell viability was reassessed with trypan blue staining (>90%) after a final cell wash and cells were prepared for staining as described below.

Lymphocyte subsets were determined by flow cytometry (FACScan, Becton Dickinson, San Jose, CA) and were expressed as percentages of 5 × 10^6 PBMCs (16). Briefly, cells were stained for 30 min on ice with optimal concentrations of fluorescein- or phycoerythrin-conjugated monoclonal antibodies: total T cells (Leu 4, anti-CD3), T helper cells (Leu 3, anti-CD4), T cytotoxic-suppressor cells (Leu 2, anti-CD8), and B cells (Leu 12, anti-CD19) (Becton Dickinson). In Study 2,
PBMCs were also stained for natural killer cells (Leu 11c, anti-CD16, phycoerythrin, IL-2R (anti-IL-2R, fluorescein), and Tkr (anti-Tkr, fluorescein) (Becton Dickinson). Because of the need to conserve cells in Study 2, PBMCs (5 × 10^5) were stained with a fluorescein- and a phycoerythrin-conjugated monoclonal antibody for two different cell types. Preliminary experiments showed no cross-reactivity between monoclonal antibodies used to stain for distinct lymphocyte subsets. Stained cells were washed in cold phosphate-buffered saline (0.1% sodium azide and 2% fetal bovine serum) and were fixed in 0.5% paraformaldehyde in preparation for acquisition and analysis.

**Statistics**

The a priori sample size calculations were based on the β-carotene group mean for DTH being 33% greater than that of the placebo group. The 33% estimated increase was based on an elderly human supplementation trial of placebo and another antioxidant vitamin, vitamin E (5). The within-group SD was 18% of the mean, based on repeated tests of DTH in healthy individuals with the same skin plant device used in the present study (17). For a sample size of 11 subjects per group, the probability of detecting a difference at the 0.05 level of significance was calculated to be 0.942 (PC-SIZE: CONSULTANT, version 1.01, by Gerard E Dallal, 1990). The type II error was equal to power of 1 to detect or 0.058.

In study 1, baseline versus posttreatment data within groups (β-carotene or placebo) were analyzed by using Student's paired t test or Wilcoxon’s rank test for data that were not normally distributed. Differences between treatment groups were analyzed by using Student’s t test for independent samples (or the Kruskal-Wallis nonparametric test for data that were not normally distributed) on mean pre- to postintervention differences (SYSTAT for the Macintosh, version 5.2; Systat Inc, Evanston, IL). The use of Student’s t test for independent samples to compare mean differences between groups is identical to testing for a significant time by treatment interaction in a repeated-measures analysis. In study 2, data were analyzed by using Student’s unpaired t test or the Kruskal-Wallis nonparametric test for data that were not normally distributed.

Two-sided 95% CIs were calculated for differences between treatment groups (SYSTAT for the PC, version 6.0). Significance was defined by a P value ≤ 0.05. Results are reported as means ± SEMs.

**RESULTS**

**Study design and subject characteristics**

In study 1 there were no significant differences in plasma β-carotene concentrations between groups at baseline; however, β-carotene supplementation for 23 d resulted in a 10-fold increase in subjects’ plasma β-carotene concentrations compared with baseline (P < 0.005). Plasma β-carotene concentrations remained constant in the placebo group. At the end of the supplementation period, the mean plasma β-carotene concentration for the β-carotene group was 11-fold higher than that for the placebo group.

Although capsule treatments were randomly assigned in the short-term study, there was a significant difference in age between those taking placebo (x̄: 65.3 y) and those taking β-carotene (x̄: 70.0 y). As a result, immune indexes were tested as dependent variables in regression equations that included treatment group and age as independent factors. There was no statistical evidence suggesting that the treatment groups would act differently on the basis of age.

Subjects experienced no significant changes in body weight for the duration of the study. Additionally, there were no significant differences in body mass index (BMI, in kg/m²) between subjects in the two treatment groups (postintervention: placebo, 25.8; β-carotene, 25.5).

Physicians supplemented with β-carotene in study 2 for 10–12 y had plasma β-carotene concentrations more than threefold higher than concentrations in men taking placebo (Table 1; P < 0.0001). No significant differences in age (placebo: 64.7 y, β-carotene: 60.8 y) or BMI (placebo: 25.3, β-carotene: 25.5) were present between placebo and β-carotene groups.

**Delayed-type hypersensitivity skin test responses**

In study 1 the pre- to postintervention change in DTH between the β-carotene and placebo groups was not significantly different. This was due to the increase in DTH response over time experienced by both treatment groups. The reason for the nonsignificant increase in the placebo group is unknown; although the applications of the DTH tests were just 3 wk apart, previous studies reported no boosting effect when using the same skin plant device (17). These increases in DTH responses were not significant for either the number of positive antigens (placebo: 25 ± 0.4 and 3.1 ± 0.6, β-carotene: 3.1 ± 0.4 and 3.5 ± 0.3, pre- and postintervention, respectively) or the sum of indurations (placebo: 11.8 ± 2.0 and 14.8 ± 3.1 mm, β-carotene: 15.7 ± 1.7 and 19.9 ± 1.4 mm, pre- and postintervention, respectively). However, there was a trend (P = 0.06) for women supplemented with β-carotene to have increased responses (sum of indurations but not number of positive antigens) to the battery of recall antigens compared with their baseline values (Figure 1). There were no significant differences in DTH responses between men supplemented long term with placebo or β-carotene (study 2; Figure 1).

**Lymphocyte proliferation**

In study 1 there was a trend toward greater lymphocyte proliferation (P = 0.07) after intervention in the β-carotene group than in the placebo group. This tendency, however, was largely the result of a nonsignificant increase in proliferative response in the β-carotene group coupled with a significant decline in lymphocyte proliferation in the placebo group over time (P < 0.05; Figure 2). The significant decline in the proliferative response of the placebo group was seen in PBMC cultures stimulated with suboptimal and optimal concentrations of ConA (5 and 50 mg/L, respectively), but not in cultures stimulated with PHA (data not shown). The specific reason for this decline in lymphocyte proliferation among those taking the placebo is unknown; nonetheless, this kind of phenomenon has been seen in previous studies (18, 19). Lymphocyte proliferation responses between men supplemented with β-carotene and men receiving placebo did not differ significantly in the long-term trial (study 2; Figure 2).
IL-2 and PGE2 production

In study 1 there were no significant differences in PHA-induced IL-2 production or PGE2 production between treatment groups after 23 d of β-carotene supplementation (Table 2). Similarly, results of ConA-induced IL-2 and PGE2 production (data not shown) were not significantly different between the placebo and β-carotene–supplemented groups.

Results of IL-2 and PGE2 production in study 2 have been reported previously in the context of their potential effects on natural killer cell activity in the elderly (20). The results are reported here in the context of T cell–mediated immunity in comparing the two β-carotene supplementation studies. ConA and PHA-induced IL-2 were produced in similar amounts in the long-term study. No significant differences were found in IL-2 production between the β-carotene and placebo groups. However, there was a trend for lower PHA-stimulated PGE2 production in the β-carotene group when compared with that produced by the placebo group (P = 0.06). There was no trend
TABLE 2
Effect of β-carotene supplementation on interleukin 2 (IL-2) and prostaglandin E₂ (PGE₂) production

<table>
<thead>
<tr>
<th></th>
<th>IL-2</th>
<th>PGE₂</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
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<tr>
<td></td>
<td>×10⁴ U/L</td>
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<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 11)</td>
<td>17.58 ± 2.9²</td>
<td>18.32 ± 3.8</td>
</tr>
<tr>
<td>β-Carotene (n = 11)</td>
<td>19.39 ± 3.6</td>
<td>17.11 ± 3.4</td>
</tr>
<tr>
<td>95% CI¹</td>
<td>NA</td>
<td>(-10.71, 4.659)</td>
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<tr>
<td><strong>Study 2</strong></td>
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<td></td>
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<tr>
<td>Placebo (n = 25)</td>
<td>NA</td>
<td>40.40 ± 6.0</td>
</tr>
<tr>
<td>β-Carotene (n = 25)</td>
<td>NA</td>
<td>34.60 ± 4.6</td>
</tr>
<tr>
<td>95% CI²</td>
<td>NA</td>
<td>(-9.370, 20.96)</td>
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</tbody>
</table>

¹ Peripheral blood mononuclear cell culture supernatants were assayed after 48 h incubation with 10 mg phytohemagglutinin/L in 10% autologous plasma. NA, not available.
² NA, not available.
³ 95% CI for group differences in the mean change from baseline to follow-up.
⁴ 95% CI for group differences in the follow-up period.

for lower ConA-stimulated PGE₂ production in the β-carotene group.

Lymphocyte subsets

Flow cytometry revealed no significant differences in total T cells (CD3+), helper T cells (CD4+), suppressor-cytotoxic T cells (CD8+), the ratio of CD4 to CD8 cells, or B cells (CD19+) between baseline and follow-up as a result of β-carotene supplementation in study 1. A comparison of postsupplementation percentages of lymphocyte subsets is presented in Table 3. In addition, complete blood count differentials were not significantly different between baseline and follow-up in numbers of white blood cells or in percentages of lymphocytes or monocytes as a result of β-carotene supplementation or placebo (data not shown).

In study 2 also there were similar percentages of total T cells (CD3+), helper T cells (CD4+), suppressor-cytotoxic T cells (CD8+), B cells (CD19+), and natural killer cells (CD16+); a similar ratio of CD4 to CD8 cells; and similar expressions of cell surface IL-2R and TfR in the placebo and β-carotene-supplemented groups when analyzed by flow cytometry (Table 3). Complete blood count differentials were not significantly different in numbers of white blood cells or percentages of lymphocytes or monocytes between the β-carotene or placebo groups (data not shown).

**DISCUSSION**

Although previous β-carotene feeding studies in animals as well as in vitro β-carotene supplementation of rodent and human cells suggested that β-carotene might enhance T cell-mediated immunity, results from in vitro and in vivo β-carotene studies have not always agreed (21). Limited studies in older individuals have suggested β-carotene-induced immunoenhancement (10); however, a need for verification of β-carotene’s involvement in immunomodulation in the elderly was necessary because of difficulty in interpreting data as a result of small sample sizes and the need to assess immune cell function.

Our short-term, high-dose β-carotene supplementation trial of longitudinal design in older women and our long-term, lower-dose β-carotene supplementation trial of longitudinal

TABLE 3
Effect of β-carotene supplementation on composition of lymphocyte subsets

<table>
<thead>
<tr>
<th></th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4:CD8</th>
<th>CD19</th>
<th>CD16</th>
<th>IL-2R²</th>
<th>TfR²</th>
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<tbody>
<tr>
<td><strong>Study 1</strong></td>
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</tr>
<tr>
<td>Placebo (n = 12)</td>
<td>75.2 ± 2.5²</td>
<td>53.7 ± 2.8</td>
<td>19.1 ± 2.1</td>
<td>3.19 ± 0.4</td>
<td>10.6 ± 1.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>β-Carotene (n = 11)</td>
<td>68.8 ± 3.2</td>
<td>49.6 ± 4.3</td>
<td>16.6 ± 2.2</td>
<td>3.95 ± 0.9</td>
<td>11.2 ± 1.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>95% CI²</td>
<td>(-7.25, 7.92)</td>
<td>(-5.63, 5.02)</td>
<td>(-2.65, 2.19)</td>
<td>(-0.35, 0.96)</td>
<td>(-1.17, 3.06)</td>
<td>—</td>
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<tr>
<td><strong>Study 2</strong></td>
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<td></td>
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<tr>
<td>Placebo (n = 27)</td>
<td>71.1 ± 1.4</td>
<td>51.3 ± 1.6</td>
<td>31.5 ± 1.6</td>
<td>1.89 ± 0.2</td>
<td>7.87 ± 0.7</td>
<td>14.8 ± 1.4</td>
<td>68.5 ± 1.5</td>
<td>64.2 ± 2.1</td>
</tr>
<tr>
<td>β-Carotene (n = 27)</td>
<td>70.4 ± 1.7</td>
<td>52.1 ± 2.1</td>
<td>30.9 ± 1.4</td>
<td>1.90 ± 0.2</td>
<td>9.08 ± 0.7</td>
<td>15.7 ± 1.4</td>
<td>68.7 ± 2.7</td>
<td>64.8 ± 2.3</td>
</tr>
<tr>
<td>95% CI²</td>
<td>(-3.80, 5.07)</td>
<td>(-6.06, 4.45)</td>
<td>(-3.66, 4.92)</td>
<td>(-0.62, 0.60)</td>
<td>(-3.21, 0.79)</td>
<td>(-4.95, 3.14)</td>
<td>(-8.58, 5.12)</td>
<td>(-7.34, 5.21)</td>
</tr>
</tbody>
</table>

¹ Values reflect postsupplementation percentages of 5000–10 000 peripheral blood mononuclear cells acquired by flow cytometry. CD3, total T cells; CD8, T cytotoxic and suppressor cells; CD4, T helper cells; CD19, B cells; CD16, natural killer cells; IL-2R, interleukin 2 receptor; TfR, transferrin receptor; NA, not available.
² Determined on subset of cohort (placebo, n = 20; β-carotene, n = 18). Values reflect activated cell-surface marker expression (48 h, 5 mg phytohemagglutinin/L).
³ NA, not available.
⁴ NA, not available.
⁵ 95% CIs are given for differences in between-group means.
design in older men both consistently showed no significant T cell immunomodulations as assessed by DTH, lymphocyte proliferation, IL-2 and PGE\textsubscript{2} production, and composition of lymphocyte subsets. Although the short-term, high-dose \(\beta\)-carotene supplementation trial showed a trend for greater lymphocyte proliferation and the long-term, lower-dose \(\beta\)-carotene supplementation trial showed a trend for lower PGE\textsubscript{2} production in the groups supplemented with \(\beta\)-carotene, the possible clinical significance of these positive trends is difficult to extrapolate.

Results from these two clinical trials in older individuals agree with two previously published studies in younger individuals (8, 9); \(\beta\)-carotene supplementation has consistently shown minimal or no effects on modulating profiles of lymphocyte subsets and T cell-mediated immunity. Murata et al (8) reported a significant increase in the ratio of CD4 to CD8 cells, with no significant individual increases or decreases in CD4+ or CD8+ T cells after 9 mo of \(\beta\)-carotene supplementation. Ringer et al (9) found no significant changes in lymphocyte profiles due to \(\beta\)-carotene supplementation (15–300 mg/d) and no significant differences in lymphocyte proliferation, IL-2 production, or quantitative immunoglobulin measurements. However, results of immunologic analyses in the trial conducted by Ringer et al were difficult to interpret because of the confounding effect of other nutrients as a result of a study design that included supplementing all treatment groups with a multivitamin and using a placebo composed of wheat germ oil, a rich source of vitamin E, a known immunomodulator (22, 23). Although Fuller et al (24) showed that \(\beta\)-carotene supplementation (30 mg/d) in young men was effective in preventing ultraviolet-light-induced suppression of DTH skin responses, overall results of \(\beta\)-carotene supplementation studies in healthy young individuals under conditions of uncompromised immunity indicate that \(\beta\)-carotene is not effective for significant T cell–mediated immunoenhancement (8, 9).

Although results of this study indicate that \(\beta\)-carotene supplementation is not effective for enhancing T cell–mediated immunity, \(\beta\)-carotene supplementation has been shown to enhance another branch of immunity in the elderly: natural or innate immunity, as measured by natural killer cell activity (20). Supplementation of elderly men (\(\geq\) 65 y) with 50 mg \(\beta\)-carotene on alternate days for 10–12 y resulted in significantly greater natural killer cell activity compared with subjects of the same age taking placebo. In the elderly, \(\beta\)-carotene supplementation may have a specificity for certain immune cell types and functions and thus be more specific to innate immune functions such as immunosurveillance of tumors and viruses.

Results from two large, randomized, placebo-controlled trials, whose subjects were primarily cigarette smokers, have raised questions concerning the safety of \(\beta\)-carotene supplementation. Because primary outcomes of these studies include incidences of cancer and cardiovascular disease, diseases that may involve T cell–mediated immunity, it is important to consider potential negative effects of \(\beta\)-carotene supplementation on T cell–mediated immune responses. Both the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Trial (25) and the Beta-Carotene and Retinol Efficacy Trial (CARET) (26) reported no benefits on cancer or cardiovascular disease of supplementation, but instead reported higher rates of lung cancer and cardiovascular disease in subjects supplemented with \(\beta\)-carotene. On the other hand, results of the Physicians’ Health Study (the cohort from which this immunologic substudy was derived) have indicated neither benefit nor harm for an average of 12 y of \(\beta\)-carotene supplementation in male physicians followed for endpoints of malignant neoplasms, cardiovascular disease, and death (27). These results held for nonsmokers, former smokers, and current smokers.

For the immunologic substudy described in this report, we selected participants from the Physicians’ Health Study who were current nonsmokers because smoking decreases concentrations of plasma \(\beta\)-carotene (28) and to maintain homogeneity of the cohort. Elderly women recruited for the short-term, high-dose \(\beta\)-carotene supplementation trial were also current nonsmokers. Although the large-scale ATBC and CARET trials were conducted largely in smokers, in light of their results, it is important to note that no adverse effects of short- or long-term \(\beta\)-carotene supplementation were observed in our analyses of T cell–mediated immunity. It was also previously reported in smokers that 14 wk of \(\beta\)-carotene supplementation (20 mg/d) showed no adverse effects on T cell–mediated immunity when in vitro PBMC cultures were incubated in autologous plasma; no change was seen in the composition of lymphocyte subsets between \(\beta\)-carotene and placebo groups, and subjects supplemented with \(\beta\)-carotene had significantly greater PHA-induced (but not ConA-induced) lymphocyte proliferation (29).

For our studies in this report, 95% CIs are given for changes between \(\beta\)-carotene and placebo groups and are consistent with \(P\) values in providing no statistical basis for rejecting the null hypotheses of equal means. In both the short- and long-term \(\beta\)-carotene trials, plasma \(\beta\)-carotene was raised to significantly higher concentrations in subjects receiving active treatment; therefore, these concentrations of \(\beta\)-carotene supplementation override any confounding effect of dietary components on plasma \(\beta\)-carotene. In addition, care was exercised to exclude subjects who took multivitamins or immunosuppressive drugs so as to eliminate confounding by extraneous micronutrients or drugs.

Although supplementation with the isolated carotenoid \(\beta\)-carotene did not result in enhanced T cell–mediated immunity in the elderly, it is important to stress the necessity of a diet that is rich in a variety of fruit and vegetables, including yellow and leafy green vegetables, which contain a spectrum of carotenoids and numerous other micronutrients, for maintenance of overall good health and prevention of chronic diseases (30–32). Components, ie, carotenoids and other micronutrients, within this kind of a healthful diet may, in the course of future research, be found to potentiate different aspects of immunity either alone or in combination.

Results of short- and long-term \(\beta\)-carotene supplementation trials in older individuals indicate that \(\beta\)-carotene supplementation is not effective for modulating the composition of circulating lymphocytes nor for enhancing T cell–mediated immunity to a significant level. In addition, no negative modulation of T cell–mediated immunity occurred in healthy elderly individuals as a result of short- or long-term \(\beta\)-carotene supplementation.

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