Natural killer cell activity in elderly men is enhanced by β-carotene supplementation\textsuperscript{1-5}

Michelle S Santos, Simin Nikbin Meydani, Lynette Leka, Dayong Wu, Nader Fotouhi, Mohsen Meydani, Charles H Hennekens, and J Michael Gaziano

ABSTRACT  Natural killer (NK) cell activity has been postulated to be an immunologic link between β-carotene and cancer prevention. In a cross-sectional, placebo-controlled, double-blind study we examined the effect of 10–12 y of β-carotene supplementation (50 mg on alternate days) on NK cell activity in 59 (38 middle-aged men, 51–64 y; 21 elderly men, 65–86 y) Boston area participants in the Physicians’ Health Study. No significant difference was seen in NK cell activity due to β-carotene supplementation in the middle-aged group. The elderly men had significantly lower NK cell activity than the middle-aged men; however, there was no age-associated difference in NK cell activity in men supplemented with β-carotene. β-carotene-supplemented elderly men had significantly greater NK cell activity than elderly men receiving placebo. The reason for this is unknown; however, it was not due to an increase in the percentage of NK cells, nor to an increase in interleukin 2 (IL-2) receptor expression, nor to IL-2 production. β-carotene may be acting directly on one or more of the lytic stages of NK cell cytotoxicity, or on NK cell activity-enhancing cytokines other than IL-2, such as IL-12. Our results show that long-term β-carotene supplementation enhances NK cell activity in elderly men, which may be beneficial for viral and tumoral surveillance.  


KEY WORDS  Natural killer cell activity, cytotoxicity, β-carotene, carotenoids, aging, natural immunity

INTRODUCTION

Natural killer (NK) cells are important in natural immunity, recognizing their viral and tumor targets without prior encounter with antigen (1). Evidence suggests that there is an important role in vivo for NK cell activity against tumors. Homozygous beige mice, genetically deficient in NK cell activity, grew tumors and developed leukemia more rapidly than their heterozygous littermates who had normal NK cell activity (1). In addition, reconstitution experiments involving the transfer of NK cells to mice or rats with little or no NK cell activity resulted in strongly acquired tumor resistance (2). Although the direct role of NK cell activity on tumors in humans is more difficult to document, people with a defective NK cell system show higher susceptibility to neoplasia, as seen in patients with Chédiak-Higashi syndrome (3) and systemic lupus erythematosus (4). Patients with no evidence of disease having high NK cell activity had a significantly longer metastasis-free survival time when compared with patients with low NK cell activity (5). On the basis of this evidence, NK cell activity has been proposed as an important immunologic component of cancer prevention. Cancer and tumor incidence increase with aging; this may be partially due to a decline in NK cell activity with aging.

Decline in immune function with aging is well established (6), a decrease in T cell–mediated function encompassing a large part of the overall decline. However, there is no clear consensus on age-related changes in innate immunity, i.e., NK cell activity. Reviews on research in NK cell activity (7, 8) show decreases, no change, and increases in NK cell activity with aging; discrepancies may be due to differences in sample size, in criteria used to define “healthy elderly,” in age ranges, and in methods. Supplementation of healthy elderly persons with micronutrients has been considered as a way to enhance immune function to optimal levels with the goal of optimizing health as one ages (9, 10).

There is some indication from human supplementation trials that β-carotene may play a role in upregulation of NK cell number and/or function. A significant increase in the percentage of CD16+ NK cells was reported in older subjects (mean age: 56 y) supplemented for 2 mo with 30–60 mg β-carotene/d. However, no measurements of the function of the lymphocytes such as NK cell activity or cytokine production were conducted, and have not been reported previously for an aging

\textsuperscript{1} From the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston; Brigham and Women’s Hospital and Harvard Medical School, Boston; the Department of Pathology, Tufts University Sackler Graduate School of Biomedical Sciences, Boston; and the Department of Medicine, Veterans Administration Medical Center, West Roxbury, MA.

\textsuperscript{2} 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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\textsuperscript{5} Address reprint requests to SN Meydani, Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: S.Meydani@hnrc.tufts.edu.

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population supplemented with β-carotene. A short-term β-carotene supplementation trial (30 mg/d for 3 mo) in men aged 35–79 y with oral leukoplakia or Barrett’s esophagus showed increases in the percentage of NK cells along with a 40% increase in NK cell activity among those supplemented with β-carotene (11). Enhancement of NK cell activity by β-carotene may be an important factor in the success of β-carotene treatment in oral leukoplakia patients, whose overall response rates to β-carotene are close to or exceed 50% (12).

A wealth of epidemiologic evidence exists showing significant inverse correlations with incidence of cancer and diets high in green leafy and yellow vegetables, in which β-carotene is a predominant carotenoid (13). Male vegetarians with serum β-carotene concentrations twice those of male omnivores showed twofold greater NK cell activity compared with omnivores; this represents one possible explanation for the decreased risk of cancer in vegetarians (14). However, whether the decreases in risk of cancer were due to the effect of β-carotene or another dietary component remain to be answered. Prospective trials examining the effects of β-carotene supplementation, such as the Physicians’ Health Study, were designed with incidence of cancer as endpoints. The Physicians’ Health Study cohort offered us a unique opportunity to examine the immunologic effects of long-term β-carotene supplementation in an aging population. We focused on NK cell activity as a functional measurement of immune reactivity relevant to one of the hypothesized outcomes of the Physicians’ Health Study, ie, cancer prevention.

SUBJECTS AND METHODS

Study population

The Physicians’ Health Study is a randomized, double-blind, placebo-controlled trial of the effect of low-dose aspirin and β-carotene (50 mg Lurotin on alternate days; BASF, Mount Olive, NJ) intakes by 22 071 US male physicians on the primary prevention of cardiovascular disease and cancer. According to a 2 × 2 factorial design, subjects were randomly assigned to receive aspirin, β-carotene, both, or neither between 1982 and 1984. The aspirin component of the study was terminated in December 1987 by the external Data Monitoring Board, primarily because the aspirin group had a highly significant (44%) reduction in the risk of a first myocardial infarction (15). The 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.

Because of the need to obtain a fresh blood specimen for isolation of lymphocytes and monocytes, a random sample of Boston area participants in the Physicians’ Health Study was recruited for this immunologic substudy. Samples for this cross-sectional comparison were collected between the months of July and November, 1993. This project was approved by the Internal Review Board of Brigham and Women’s Hospital and Harvard Medical School, and the approval was acknowledged by the Human Investigation Review Committee of Tufts University Health Sciences and New England Medical Center Hospitals. Informed, written consent was obtained from all participating physicians.

Recruitment was done by telephone in a blinded manner, with the goal of enrolling equal numbers of physicians from each coded treatment group. At the time of recruitment, subjects were asked to refrain from taking aspirin or other nonsteroidal antiinflammatory drugs 10 d before the blood draw. Subjects known to be taking extraneous (nonstudy) vitamin supplements were excluded during recruitment to avoid confounding results. Blood samples from fasted subjects in both treatment groups and age groups were collected randomly at the physicians’ residence or place of employment between 0630 and 1030. Of 73 Boston area physicians participating, 14 were excluded: 6 because they had taken aspirin within 72 h of the blood draw, 7 because they had used supplements not provided by the Physicians’ Health Study, and 1 in the β-carotene–supplemented group because of a low plasma β-carotene concentration.

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 one to two medications, and only 7% took three medications, the majority being antihypertensive and cholesterol-lowering drugs. On the basis of questionnaire results at the time of the blood draw, physicians were in general good health.

Compliance with supplement intake for 2 mo before blood collection was self-reported as part of the questionnaire: 87% of the subjects reported taking all their pills and 8.6% missed one to three pills. Records of average annual dietary intakes of vitamin A and carotenoid-rich foods were collected by mail with a partial Willett food-frequency questionnaire; however, the dietary data are unavailable, pending completion of the Physicians’ Health Study. Nonetheless, the amount of β-carotene given (50 mg on alternate days) was many times greater than the average dietary intake of β-carotene by American men (0.5–6.5 mg/d) (16). This suggests that it is unlikely that the average dietary β-carotene intake could significantly enhance β-carotene status more than intakes achieved by supplementation. This was confirmed by assessment of plasma and peripheral blood mononuclear cell (PMBC) β-carotene concentrations by HPLC (17).

Physicians were stratified into middle-aged (51–64 y; mean: 57 y) and elderly (65–86 y; mean: 74 y) groups, based on previous research findings and suggested guidelines of gerontologic research, which indicate that “elderly” refers to individuals ≥65 y old (18).

Blood collection and lymphocyte isolation

Subjects were asked to fast the night before the blood draw. Blood was collected in foil-wrapped evacuated tubes with sodium heparin, and was maintained at room temperature until processed. All laboratory blood work was done under red light to minimize ultraviolet light exposure and decomposition of β-carotene. PBMCs were isolated from whole blood by using Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation. The buffy layer was isolated and washed twice in complete medium: RPMI 1640 (BioWhittaker, Walkersville, MD) with 100 000 U penicillin and streptomycin/L (Gibco BRL, New York), 2 mmol glutamine/L (Gibco BRL), and 25 mmol Hepes/L (Sigma, St Louis). Trypan blue staining was used to assess cell viability, and lymphocytes were counted in a hemocytometer under a light microscope.
Determination of the percentage of NK cells

The percentage of NK cells was determined by flow cytometry (FACscan; Becton Dickinson, San Jose, CA) from 5 × 10⁵ PBMCs in cold phosphate buffered saline (0.1% sodium azide and 2% fetal bovine serum; FBS) after a 30-min incubation on ice with 10 µL phycoerythrin-conjugated monoclonal antibody Leu11c/anti-CD16 (Becton Dickinson). This method was described previously in greater detail (19).

NK cell activity

K562 human myelogenous leukemia cells (generous gift from the laboratory of Judy Lieberman, Tufts University, Boston) were cultured in complete medium as described above with 10% FBS (Gibco BRL, New York) and used for assays in their log phase. FBS was chosen over human AB serum because preliminary testing showed that the type of serum did not have an effect on the results in our system. Targets were labeled with ⁵¹Cr (25 µL × decay factor/10 × 10⁶ cells; DuPont NEN, Boston) and allowed to incubate 60–90 min to reduce spontaneous release of chromium; 1 × 10⁶ targets/well were added to appropriately diluted PBMC preparations (1 × 10¹⁰ cells/L) in triplicate (flat-bottomed, 96-well microtiter plates; Becton Dickinson and Co, Lincoln Park, NJ) for final effector to target cell ratios (E:T) of 12.5:1, 25:1, 50:1, and 100:1. Effectors and targets were incubated for 4 h after centrifugation at 18 × g for 5 min. Spontaneous release was assessed as the amount of ⁵¹Cr released by target cells alone (six wells) and maximum release was assessed by the amount of ⁵¹Cr released through three cycles of a 30-min freeze at −70 °C and a 45-min thaw at room temperature. This maximum release method was chosen over incubation in sodium dodecyl sulfate detergent because the freezing and thawing resulted in substantially greater maximum release values. Supernates from each well (50 µL) were spotted onto thick mats and sealed in plastic bags for gamma counting in a chromium window on the 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland). Spontaneous release remained below 10% except on two occasions during which it increased slightly to 15–20%. Percentage specific lysis is defined as follows: (experimental cpm – spontaneous cpm) / (maximum cpm – spontaneous cpm) × 100.

To reduce the NK cell cytotoxicity data to a single value that is meaningful in terms of lytic activity, lytic units (LU), the number of PBMCs required to cause a defined amount of target cell lysis—30% in this study—were calculated. Data were reduced to linearity and LUs were defined by a simple exponential fit equation (20): y = 1 – e⁻ᵏ⋅x, where y is cell-mediated ⁵¹Cr release, x is E:T, and k is the slope obtained by plotting the logarithm (A – y), where A = 100, the maximum of the dose-response curve.

IL-2 and prostaglandin E₂ production

A standard bioassay using the human recombinant IL-2–sensitive CTLL-2 cell line (developed in our lab from CTLL cells in rat-conditioned medium; ATCC, Rockville, MD) was used to determine the amount of IL-2 produced by 48-h PBMC cultures (1.111 × 10⁹ cells/L) stimulated with either phytohemagglutinin (PHA, 10 mg/L) or concanavalin A (10 mg/L) according to the method of Gillis et al (21). This method was described in detail previously (22). Prostaglandin E₂ (PGE₂) production was measured by RIA (using the same culture conditions as IL-2) as described previously (22). The PGE₂ antibody (polyclonal rabbit γ-globulin) was kindly provided by J Dupont (Iowa State University, Ames) and M Mathias (Colorado State University, Fort Collins).

Statistics

Data were analyzed by using unpaired Student’s t test and two-way analysis of variance (ANOVA) general linear models with age group (middle-aged or elderly) and treatment group (placebo or β-carotene) serving as independent factors (SYSTAT for the Macintosh, version 5.2; Evanston, IL). Data that were not normally distributed were analyzed by using the Kruskal-Wallis nonparametric Mann-Whitney U test. Levene’s (23) test of heterogeneity of variance was performed for variables suspected of unequal variance; significance at the 0.05 level resulted in our using the separate-variance P value instead of the pooled-variance P value. Results are reported as x ± SEM.

RESULTS

Subject characteristics at the time of the blood draw are shown in Table 1. There were no significant differences in mean age or body mass index (BMI; in kg/m²) between treatment groups, although middle-aged placebo subjects tended to weigh more than elderly placebo subjects. Plasma β-carotene in the supplemented group was significantly elevated to concentrations more than three times those of the placebo group (P < 0.0001). The PBMC β-carotene content was significantly higher in the β-carotene group than in the placebo group (P < 0.05). No significant age differences were seen in PBMC β-carotene concentrations. Although under fairly tight homeostatic control, plasma retinol concentrations were measured as a first step in trying to determine whether the effect of β-carotene may be mediated through retinoid metabolites, particularly because retinoids added in vitro to murine cell cultures resulted in upregulated IL-2–induced NK cell activity (24). Within the placebo group, plasma retinol concentrations in the middle-aged men were significantly greater than in the elderly men. No significant effect of β-carotene supplementation on plasma retinol concentration was observed in either age group. Plasma α-tocopherol concentrations were not significantly different, regardless of treatment or age group; however, the elderly placebo group tended to have greater PBMC α-tocopherol concentrations than the middle-aged group (P = 0.08, data not shown). A complete profile of plasma carotenoids and tocopherol and peripheral blood cell β-carotene and α-tocopherol was presented in a separate report (25); no significant differences in other PBMC or plasma carotenoids were seen between treatment groups.

NK cell cytotoxicity as a percentage of specific lysis for four different E:T ratios is shown in Figure 1. Elderly (65–86 y) placebo subjects had significantly lower NK cell activity than middle-aged (51–64 y) placebo subjects. However, long-term β-carotene supplementation maintained NK cell activity in the elderly at levels comparable with those of middle-aged subjects, so that there was no significant age difference in NK cell activity. In turn, when NK cell activity was compared by treatment group within age categories, no significant β-carotene effect was seen among...
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Age</th>
<th>BMI</th>
<th>Plasma β-carotene</th>
<th>PBMC β-carotene</th>
<th>Plasma retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>kg/m²</td>
<td>μmol/L</td>
<td>pmol/10⁷ cells</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle-aged (n = 17)</td>
<td>57 ± 1</td>
<td>25.2 ± 0.9</td>
<td>0.56 ± 0.08</td>
<td>11.2 ± 1.8</td>
<td>2.41 ± 0.13⁷</td>
</tr>
<tr>
<td>Elderly (n = 13)</td>
<td>75 ± 2</td>
<td>25.5 ± 1.0</td>
<td>0.57 ± 0.09</td>
<td>19.6 ± 5.0</td>
<td>2.01 ± 0.08</td>
</tr>
<tr>
<td>β-Carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle-aged (n = 21)</td>
<td>56 ± 1</td>
<td>25.8 ± 0.7</td>
<td>1.6 ± 0.21⁴</td>
<td>66.1 ± 12.8¹</td>
<td>2.22 ± 0.10</td>
</tr>
<tr>
<td>Elderly (n = 8)</td>
<td>72 ± 2</td>
<td>24.7 ± 1.0</td>
<td>1.8 ± 0.22⁶</td>
<td>70.4 ± 24.9⁷</td>
<td>2.34 ± 0.24</td>
</tr>
</tbody>
</table>

¹̅x ± SEM.
² Placebo (middle-aged, n = 14; elderly, n = 12) and β-carotene (middle-aged, n = 14; elderly, n = 4).
³ Significantly greater than elderly placebo, P < 0.05 (Kruskal-Wallis nonparametric test).
⁴ Significantly greater than middle-aged placebo group (unpaired t test of log-transformed data): ⁴ P < 0.0001, ⁵ P < 0.005.
⁶,⁷ Significantly greater than elderly placebo group (unpaired t test of log-transformed data): ⁶ P < 0.0001, ⁷ P < 0.05.

the middle-aged subjects. However, β-carotene supplementation in the elderly produced significantly greater NK cell activity when compared with placebo subjects of the same age (P < 0.05). ANOVA showed a trend for a treatment-by-age interaction (P = 0.08), supporting our age group stratification.

Because the ⁵¹Cr release assay depends substantially on the state of the target cells, interassay variability may be introduced when multiple days are required to analyze a large number of subjects. In an attempt to standardize multiple assays and express the results in terms of units of NK cell lytic activity, the data indicating percentage specific lysis at multiple E:T ratios have been reduced mathematically to a single value and are shown in Table 2. Results of NK cell cytotoxicity expressed in LUs are comparable with those described above for Figure 1.

Flow cytometry revealed no significant changes in the percentage of CD16⁺ NK cells as a result of β-carotene supplementation, regardless of age group (data not shown). Doubly stained CD16⁺CD56⁺ NK cells as well as CD56⁺ single-stained NK cells were analyzed at a later date on a subset of elderly physicians (n = 12) who had continued their supplementation uninterrupted, confirming no significant differences in percentages of NK cells as a result of β-carotene supplementation (data not shown).

The production of two different modulators of NK cell activity—the enhancing protein cytokine IL-2 and the suppressive arachidonic acid derivative PGE₃—are shown in Table 3. No significant differences were found in IL-2 production as a result of β-carotene supplementation or age category, nor were there any significant differences in PGE₃ production.

There was a significant inverse correlation between NK cell activity and age for all four E:T ratios as well as for LUs (P < 0.05). A representative correlation (r = -0.289) at an E:T of 25:1 is shown in Figure 2. No significant correlations were found between NK cell activity and plasma β-carotene or NK cell activity and PBMC β-carotene. Neither were there significant correlations between the percentage of NK cells and age, supporting the flow cytometry data.

DISCUSSION

This study represents the first analysis of the effects of long-term β-carotene supplementation on NK cell activity in a healthy aging population. Supplementation of elderly subjects with 50 mg β-carotene on alternate days resulted in significantly greater NK cell activity compared with placebo subjects of the same age. Furthermore, NK cell activity among the supplemented elderly subjects was maintained at a level comparable with that of their younger, middle-aged counterparts, in contrast with elderly males (65–86 yr) taking placebo, who had significantly lower NK cell activity than middle-aged men.

Some investigators who have seen increases in NK cell activity have found concomitant increases in the percentage of CD16⁺ NK cells, which correlated well with NK cell activity and in part explained the increased cytotoxicity (26, 27). We also saw a significant positive correlation between the percentage of CD16⁺ NK cells and NK cell activity (r = 0.471, P = 0.002). However, an increase in the percentage of NK cells did not serve as a contributing factor to the enhanced NK cell activity seen in our elderly β-carotene–supplemented group, nor did a decrease in the percentage of NK cells in elderly subjects taking placebo contribute to their lower NK cell activity. Our findings contrast with those of Watson et al.’s (28), showing an increase in the percentage of NK cells after short-term β-carotene supplementation of older individuals; however, each group contained only two women and two men, which could contribute to difficulty in data interpretation.

Three months of β-carotene supplementation (30 mg/d) of subjects with oral leukoplakia or Barrett’s esophagus resulted in a significant increase in their percentage of NK cells (11); differences in percentages of NK cells between their subjects and our subjects may have been due to the length of β-carotene supplementation and/or to the subjects’ health.

IL-2, a major stimulator of the clonal expansion of NK cells, did not contribute to the increased NK cell activity seen among the β-carotene–supplemented elderly subjects because no significant differences in IL-2 production were detected as a result of β-carotene supplementation. This finding agrees with the absence of change in IL-2 production that we observed when elderly women were supplemented with a higher dose of β-carotene (90 mg/d) for a short period of time (3 wk) (29).

Although greater cell-surface IL-2 receptor (IL-2R) expression could contribute to increased NK cell activity via IL-2–induced upregulation of NK cell proliferation, PBMC surface expression...
of IL-2R after β-carotene supplementation was not significantly induced over that of placebo after 48 h of stimulation with the optimal PHA concentration (5 mg/L). Our results are in contrast with those of Prabhala et al. (30), which showed a significant increase in the percentage of PBMCs expressing IL-2Rs after 72 h of in vitro β-carotene supplementation (100 nmol/L). Possible reasons for the discrepancy in results are the fact that Prabhala et al. (30) measured the ability of in vitro β-carotene to effect IL-2R expression on resting PBMCs (no mitogenic stimulation), whereas we measured the ability of in vivo β-carotene to upregulate IL-2R expression while PBMCs were undergoing PHA-induced clonal expansion.

The fact that our study did not show an increase in IL-2 production or IL-2R expression as a result of β-carotene supplementation agrees with our finding of no increase in the percentage of CD16+ NK cells because immunostimulation via IL-2 or IL-2R would have resulted in the clonal expansion of NK cells. Although IL-2 is an unlikely candidate for upregulation of NK cell activity by β-carotene in elderly persons, it remains to be determined whether or not other NK cell-stimulating cytokines such as tumor necrosis factor α (TNF-α), interferon α, or interferon γ (IFN-γ) may contribute to the β-carotene–induced enhancement of NK cell activity seen among the elderly.

Mechanisms of direct enhancement of NK cell activity by β-carotene in elderly persons through upregulation of important NK cell proteins involved in target cell lysis, such as perforin and granzymes (serine esterases), are also under investigation based on animal studies of age-associated decreases in these NK cell lytic proteins. Perforin was found to be reduced in cytotoxic T lymphocytes (CTL) from aged compared with young mice, and granzyme A was also diminished in sensitized spleen cells from aged compared with young mice (8). Furthermore, messenger RNA concentrations of serine esterase and perforin declined with age in alloantigen-stimulated CTL (8). Based on the murine model and the fact that cytotoxic mechanisms of CTL and NK cells parallel one another, it is possible that one or more of these declines in lytic machinery occur with age in humans and may be reversed by the action of β-carotene.

Although the exact mechanism remains to be elucidated, we have shown that long-term, low-dose β-carotene supplementation enhanced NK cell activity in healthy elderly men when compared with placebo and healthy elderly men maintained their NK cell activity at levels comparable with those of middle-aged subjects.

### Table 2

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Lytic activity $\text{LU}_{50}/10^6$ PBMC $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>156.3 ± 37.7 $^3$</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>39.8 ± 6.7</td>
</tr>
<tr>
<td>Elderly</td>
<td>102.1 ± 23.8</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>63.5 ± 7.4 $^4$</td>
</tr>
<tr>
<td>Elderly</td>
<td></td>
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</table>

$^1 \overline{x} \pm \text{SEM}$. $^2$ Lytic unit (LU) = the number of peripheral blood mononuclear cells (PBMC) required to cause 30% target cell lysis. $^3$ Significantly greater than elderly placebo group, $P < 0.05$ (unpaired t test on log-transformed data).

### Table 3

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>IL-2 $\times 10^4$ U/L</th>
<th>PGE$_2$ nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>37.3 ± 6.1</td>
<td>2.64 ± 0.5</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>44.6 ± 9.8</td>
<td>3.53 ± 1.4</td>
</tr>
<tr>
<td>Elderly</td>
<td>32.9 ± 5.4</td>
<td>1.56 ± 0.3</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>39.0 ± 8.4</td>
<td>2.05 ± 0.6</td>
</tr>
<tr>
<td>Elderly</td>
<td>39.0 ± 8.4</td>
<td></td>
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</tbody>
</table>

$^1 \overline{x} \pm \text{SEM}$. Cultures stimulated by using 10 mg phytohemagglutinin/L for 48 h. IL-2, interleukin 2; PGE$_2$, prostaglandin E$_2$. 
In contrast, elderly placebo subjects had significantly lower NK cell activity than middle-aged subjects. Although further confirmation is required, our data raise the possibility that increased intake of β-carotene by elderly individuals may be beneficial for enhancing NK cell activity, potentially increasing viral and tumoral surveillance and providing one possible link between β-carotene intake and prevention of certain cancers.

We express our sincere appreciation to all participating physicians from the Physicians’ Health Study, without whose dedicated collaboration this project would not have been feasible. Many thanks are also due to Joanne Karimbakas, Jennifer Leka, and Sarah Byrne for their enthusiastic participation in our mobile sample collection; to Tom McFarland for his advice on establishing the target cell line; to Susan Agger for her expertise in flow cytometry; and to Adrienne Bendich for critical review of this manuscript.

REFERENCES


FIGURE 2. Inverse correlation between natural killer cell activity and age (n = 56). O, β-carotene group; □, placebo group. A ratio of effector to target cells (E:T) of 25:1 is shown, representative of similar significant results for all E-T values and lytic units. Pearson’s r = −0.289, P < 0.05.