Vitamin E Supplementation Improves Cell-Mediated Immunity and Oxidative Stress of Asian Men and Women

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ABSTRACT Vitamin E is an efficient antioxidant and a modulator of the immune system. Although racial differences in both baseline vitamin E level and immunologic subsets are known, no reliable data exist for the Asian population. Furthermore, the extent of the effect of α-tocopherol in protecting lymphocyte cells against oxidative stress and its association with cell-mediated immunity have not been elucidated. This study was undertaken to investigate the immunologic and antioxidant effects of vitamin E in healthy ethnic Chinese men and women. Volunteers < 35 y old (n = 26) were supplemented with 233 mg/d dl-α-tocopherol for 28 d. The in vitro proliferative response to phytohemagglutinin (PHA) or lipopolysaccharide (LPS) of T-lymphocytes was determined in the study group before and after vitamin E supplementation. Cell-mediated immunity subsets and hydrogen peroxide production in T-lymphocytes were investigated by flow cytometry. The oxidant-antioxidant balance in plasma and urine was studied by spectrophotometric and gas chromatography-mass selective detection methods. The antioxidant properties of vitamin E were established (P < 0.01) by the elevation of plasma vitamin E, together with depression in both plasma malondialdehyde and urinary DNA adduct 8-hydroxy-2′-deoxyguanosine after supplementation. Our data suggest a specific requirement for vitamin E in total-T and T-helper cell proliferation. We present the first evidence of the beneficial effects of supplemental vitamin E in healthy Chinese individuals on cell-mediated immunity and oxidative stress. J. Nutr. 130: 2932–2937, 2000.

KEY WORDS: • vitamin E • free radicals • immunity • oxidative stress • humans

Many chronic diseases such as coronary heart disease, cancer, and inflammatory and neurological disorders have been associated with the action of free radicals (Di Mascio et al. 1991, Freeman and Crapo 1982, Rumin et al. 1993). Free radicals such as the superoxide anion, the hydroxyl radical, hydrogen peroxide (H₂O₂) and singlet oxygen can be generated in vivo by exogenous factors such as imbalances in the diet, tobacco smoke, pollutants and other toxins, or from endogenous sources such as lipid peroxidation, inflammation, secondary lesions and biochemical reactions (Di Mascio et al. 1991, Freeman and Crapo 1982, Slater et al. 1987). The long-term presence of these oxidizing species will eventually detrimentally affect the human body (Yu 1994).

Free radicals are also produced by cells of the immune system. The purpose of such an immune cell response is to destroy invading organisms. However, strong oxidants such as oxygen free radicals impose additional stress on the immune system, resulting in a diminished response against invaders (Calder and Newholme 1993, Di Mascio et al. 1991, Goodwin and Garry 1983, Prasad 1980, Prince et al. 1985, Roy et al. 1991).

The body has sophisticated antioxidant defense systems including enzymes and vitamins. These protect against free radical damage either directly or indirectly. Vitamin E (α-tocopherol) is a lipid-soluble vitamin that is essential for cellular growth and maintenance of membrane permeability (Burton et al. 1982). It is also an efficient free radical quencher, thereby influencing atherogenesis and atherosclerosis (Eisterbauer et al. 1989, Rumin et al. 1993). Nevertheless, the extent of α-tocopherol’s protection of lymphocytes against oxidative stress and its association with cell-mediated immunity in healthy individuals have not been well studied. The role of vitamin E in lymphocyte function seems to be of importance because its concentration was 10 times higher in these bodies than in red blood cells (Hatam and Kayden 1979).

In vitamin-deficient elderly populations, vitamin E supplementation at 400 or 800 μg/d for 30 d increased lymphocyte proliferation and interleukin-2 (IL-2) production and reduced serum lipid hydroperoxides (Meydani et al. 1990). In adults with infectious diseases, supplementation with >135 mg/d (200 μg/d) α-tocopherol showed a significant positive correlation with the T-helper/T-suppressor (CD4/CD8) ratio (Chavance et al. 1985). Adachi et al. (1997) reported that vitamin E supplementation at 100 mg/d raised natural killer (NK) cell activity in young boys suffering from Shwachmann’s syn...

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drome, which is characterized by impaired NK cell activity. In healthy men, supplementation of α-tocopherol at 200 mg/d for 8 wk helped to prevent oxidative stress induced by dietary polyunsaturated fatty acids as well as to restore lymphocyte proliferative response to concanavalin A (Kramer et al. 1991). In separate studies, the incorporation of α-tocopherol into human lymphocyte cultures reversed immunosuppression induced by lipid peroxidation in the presence of mitogens (Hoffeld 1981 and 1983). The mechanisms by which vitamin E influences cell proliferation may be related to its ability to quench free radicals formed as mitogen-induced products of the lipooxygenase pathway, derived from arachidonate metabolism and phospholipid turnover (Freeman and Crapo 1982).

In several world regions, the practice of vitamin E supplementation has gained popularity among healthy working individuals. However, there are no data regarding its efficacy or dose effectiveness on immune functions in the Asian population. In healthy individuals, a lower level of activated T-helper/inducer cells (CD4) and T-suppressor/cytotoxic cells (CD8) have been reported in Asian (Chinese, Japanese, and others) populations compared with Caucasians (Prince et al. 1985).

Although racial differences for both baseline plasma vitamin E concentration and the distribution of T-cell subsets have been investigated, there is still a need to understand whether a correlation exists between free radical involvement and immunity enhancement. Using a flow cytometer, we measured the lymphocyte subsets, namely, T cells (CD3), T-helper/inducer cells (CD4), T-suppressor/cytotoxic cells (CD8), NK cells (CD56), IL-2 receptor (CD25) and the oxidative stress (as H2O2) of the T-lymphocytes. Other biological variables such as total plasma cholesterol and α-tocopherol concentrations were also measured. The oxidant status of the subjects, expressed as malondialdehyde and urinary α-tocopherol concentrations, were investigated. The oxidative stress of the fresh lymphocytes and α-tocopherol concentrations in blood and urine samples of the participants, who had fasted overnight, were established, each individual was provided with gel capsules containing 233 mg vitamin E (400 IU), which was sufficient to improve delayed-type hypersensitivity (DTH) and to increase proliferation of lymphocytes in vivo.

**SUBJECTS AND METHODS**

**Recruitment and assignments of the subjects.** Healthy men (n = 13) and women (n = 13) ranging in age from 25 to 35 y were recruited from government and private offices and from The University of Hong Kong. The subjects were informed of the objective and design of the study and the level of commitment required. All subjects had to sign a consent form approved by the University of Hong Kong Ethics Committee. The criteria for selection of the experimental subjects were as follows: 1) nonsmokers, 2) no gastrointestinal disorders or diarrhea within one week before the study, 3) no history of chronic diseases, and 4) no consumption of any health supplements, oral contraceptives, hypolipidemic drugs, thyrxyine, estrogen or nonsteroidal anti-inflammatory drugs for the past 6 mo. Furthermore, those individuals whose blood pressure and body mass index exceeded 120/80 mm Hg and 27 kg/m², respectively, were excluded from the study. These criteria are fundamental because lymphocyte subsets, degenerative diseases and oxidative DNA damage can be influenced by smoking, gender differences, body mass index and stress, among others (Loff et al. 1992).

Before subjects started vitamin supplementation, early-morning blood and urine samples of the participants, who had fasted overnight, were taken to obtain the necessary baseline levels. After these values were established, each individual was provided with gel capsules containing 233 mg vitamin E (400 IU) as α-tocopherol, Nature’s Way, R.P. Scherer, Victoria, Australia). They were instructed to take a daily dose after dinner for 28 d. At the end of the 28-d supplementation period, blood and urine samples were taken once again. The vitamin E supplementation dose used was adopted from the method of Meydani et al. (1990 and 1998) who reported that vitamin E supplementation of 266 mg/d (400 IU/d) or 523 mg/d (800 IU/d) for 30 wk was sufficient to increase proliferation of lymphocytes in vivo.

**Blood samples.** Blood from subjects who had fasted overnight was collected into EDTA tubes and processed immediately for isolation of lymphocyte and plasma by density centrifugation using a Ficoll-Paque plus centrifuge (Pharmacia Biotech, Uppsala, Sweden). The isolated lymphocytes were divided into two parts. One part was used for immunophenotyping of lymphocyte subsets and measurement of total cellular H2O2 production by flow cytometry, the other part was tested for the proliferative response of the lymphocytes to a 48-h challenge of the mitogens phytohemagglutinin (PHA) and lipopolysaccharide (LPS) (Sigma Chemical, St. Louis, MO). The plasma was stored at -20°C and analyzed later for α-tocopherol, total cholesterol and malondialdehyde (MDA; as a free radical indicator).

**Determination of proliferative response to PHA and LPS.** Viability and proliferative responses of lymphocytes in culture medium 1640 RPMI containing inactivated fetal bovine serum, 1 x 10^7 U/L penicillin G sodium, 100 mg/mL streptomycin and 100 mg/mL fungizone (Gibco BRL, Life Technologies Grand Island, NY) were measured by 0.4% trypan blue (Sigma) exclusion after 48 h at 37°C, 5% CO2. 95% humidity in the presence or absence of 2.5 mg/L PHA of the P-form, PHA-P (Sigma), or 5.0 mg/L LPS (Escherichia coli, D127: BB strain, Sigma).

**Oxidative stress determination of H2O2 production by flow cytometry.** Oxidative stress of the fresh lymphocytes was determined before and after vitamin E supplementation by capturing the fluorescent emission of the cells conjugated to 2′,7′-dichlorofluorescent diacetate (H2DCFDA; Molecular Probes, Eugene, OR) according to the method of Robinson and Plocki (1991). To 2 x 10^7 of lymphocyte suspension, 1 μL of 20 mmol/L H2DCFDA was added and incubated at 37°C for 15 min. For the positive control group, H2O2 production was induced by the addition of 100 μg/mL phorbol myristate acetate (PMA; Sigma) to the lymphocyte suspension for 5 min. The rate of H2O2 production from fluorescent lymphocytes was determined by flow cytometry (Coulter Epics, Miami, FL) at 488 nm excitation and 530 nm emission. The amount of H2O2 produced by the lymphocytes was determined as the mean fluorescent intensity of 10,000 cells counted by the flow cytometer. The concentration of the fluorescent cells was recorded as a logarithm and was expressed as an arbitrary unit.

**Immunophenotyping by flow cytometry.** Immunophenotyping was conducted in fresh lymphocytes before and after vitamin E supplementation. The cell concentration was adjusted in PBS to 1 x 10^7/L. The lymphocyte subsets (total-T, T-helper/inducer, T-suppressor/cytotoxic, NK cell, IL-2 receptor) were immunophenotyped with dual-colored conjugated monoclonal antibodies markers CD3, CD4, CD8, CD56 and CD25, respectively (PharMingen, San Diego, CA) according to the method of Landay et al. (1991). The total-T CD3 subset was conjugated with fluorescein isocyanate (FITC); the other subsets were conjugated with phycoerythrin (PE). In brief, 20 μL of monoclonal antibody was added to 100 μL of 1 x 10^7/L cells. The mixture was incubated in the dark for 30 min and then washed with PBS with 20 g/L bovine serum albumin (Sigma) and 10 g/L sodium azide (Merck, Dramaort, Germany). The cells were resuspended in cold PBS and analyzed by flow cytometry using dual-color compensation. Before analysis, the flow cytometer was optimized and aligned with DNA beads (Coulter, Miami, FL) and isotypic control immunoglobulin (IgG2a/FITC/IgG1-PE) (PharMingen). The corresponding data of 10,000 events per sample were acquired. The lymphocyte population was gated on the flow cytometer and the data were analyzed using the ModFit Winlist software (Verity Software House, Topsham, ME).

**Plasma α-tocopherol and cholesterol.** Spectrophotometric assays were adapted from Mezzetti et al. (1995) for plasma α-tocopherol determination. In brief, 1.0 mL of absolute ethanol and 2.0 mL of heptane were added to 1.0 mL of plasma. The sample was mixed and centrifuged at 1500 x g for 5 min. To 1.0 mL of supernatant, 0.2 mL of 1.47 g/L bathophenanthroline (Sigma), 0.1 mL of 0.4 g/L iron (III) chloride and 0.1 mL of 62.3 g/L phosphoric acid were added and mixed for a few minutes before spectrometric measurement in a
Spectrophotometric UV/Visible Spectrophotometer UV-160A (Shimadzu, Tokyo, Japan). Total cholesterol was measured by Cholesterol 20 (±352.20, Sigma).

**Plasma lipid oxidation.** Oxidized plasma lipids were measured as plasma MDA according to the method of Harats et al. (1990). To 1.0 mL of plasma, 0.5 mL of 350 g/L trichloroacetic acid (TCA), and 1.0 mL of 0.5% thiobarbituric acid were added and mixed. The mixture was incubated at 60°C for 90 min. After cooling at room temperature, 1.0 mL of 700 g/L TCA and 2.0 mL of chloroform were added, mixed and centrifuged at 1500 × g for 20 min. The absorbancy of the sample supernatant was measured at 532 nm.

**8-Hydroxy-2'-deoxyguanosine as a urinary biomarker of oxidative DNA damage.** Oxidative DNA damage can be assessed in humans in vivo from the urinary excretion of the DNA-repair product 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Loft et al. 1992, Tagesson et al. 1995). In our study, urine was collected before and after vitamin E supplementation and the DNA adduct was determined using a gas chromatograph provided with a mass selective detector (GC-MSD). According to the method of Dizdaroglu (1991), 8-OHdG adduct was derived from dried urine samples by heating with bis-(trimethylsilyl)-trifluoroacetamide (containing 1% trimethylchlorosilane, Sigma). Using the internal standard 2'-deoxyadenosine (Sigma), the peak area ratio of 8-OHdG was measured by GC-MSD with selective ion monitoring. The concentration of 8-OHdG was determined by a standard curve constructed with a peak/area ratio of 8-OHdG of UV radiation-damaged calf thymus DNA in formic acid (Sigma) to 2'-deoxyadenosine.

**Statistical analysis.** Results are expressed as means ± SEM. For paired group analysis, one-sample Student’s t test for paired data was used to determine the significance of differences between pre- and post-E supplementation for plasma α-tocopherol, cholesterol, malondialdehyde, T cells and subsets, and urinary 8-OHdG. A 3 × 2 factorial design was used for multiple comparisons of lymphocyte hydrogen production; two-way ANOVA for multiple comparisons was used for the lymphocyte proliferation study. The level of statistical significance was set at P < 0.05 for multiple comparisons. All analyses were performed on a personal computing system using StatView 5.0 for the Macintosh (Abacus Concepts, Berkeley, CA).

**RESULTS**

**Plasma α-tocopherol and cholesterol.** There were no dropouts and none of the participants reported any side effects during the experiment. Their plasma vitamin E concentrations almost doubled (P < 0.01) after vitamin E supplementation (Table 1). Their baseline plasma total cholesterol concentration was <5.0 mmol/L and vitamin E supplementation had no effect. There were no differences in plasma vitamin E or cholesterol between men and women before or after vitamin E supplementation.

**Oxidative stress variables.** There were no significant differences between men and women in plasma MDA and urinary 8-OHdG levels before and after vitamin E supplementation (Table 1). Vitamin E treatment for 28 d significantly lowered urinary 8-OHdG (P < 0.001) and plasma MDA (14%, P < 0.001) of the healthy subjects. Urinary 8-OHdG was 19.30 mmol/L before vitamin E supplementation, comparable to that of normal healthy individuals who excreted 14.9 mmol/L of urinary 8-OHdG (Tagesson et al. 1995).

Before supplementation, lymphocytes treated with PMA had 40% greater oxidative stress compared with untreated cells. The effectiveness of vitamin E as an antioxidant was further demonstrated by the significant 44% reduction in lymphocyte H₂O₂ production after vitamin E supplementation in both PMA-treated and untreated cells (P < 0.01) (Table 2).

**DISCUSSION**

The viability of the lymphocyte culture ranged from 90 to 94%. The proliferation of lymphocytes in culture was not affected by PHA but was significantly greater in the presence of LPS (P < 0.05) (Fig. 1). The weak mitogenic effect of PHA was explained in subsequent experiments (data not shown) showing that the PHA-P used in this study exhibits a much weaker mitogenic effect than PHA-M (Sigma) at any given dose. Supplementation with vitamin E significantly increased lymphocyte proliferation both in the presence and absence of mitogen challenge (P < 0.01).

Table 3 presents the distribution of various lymphocyte subsets before and after vitamin E supplementation. Immunophenotyping of the lymphocyte subsets identified the enhanced proliferation of total-T, T-helper/inducer cells (P < 0.01) and the CD4/CD8 ratio after vitamin E supplementation in both men and women. Both NK cells and IL-2 receptors were unaltered by vitamin E supplementation.

**TABLE 1**

The effect of vitamin E supplementation on plasma α-tocopherol (TOC), cholesterol, and malondialdehyde and urinary 8-hydroxy-2'-deoxyguanosine (U8HD) in Asian men and women

<table>
<thead>
<tr>
<th></th>
<th>Before vitamin E supplementation</th>
<th>After vitamin E supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 26)</td>
<td>Male (n = 13)</td>
</tr>
<tr>
<td>TOC, mmol/L</td>
<td>14.25 ± 0.56</td>
<td>13.74 ± 0.68</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>3.16 ± 0.08</td>
<td>3.34 ± 0.10</td>
</tr>
<tr>
<td>U8HD, μmol/L</td>
<td>19.03 ± 1.61</td>
<td>18.27 ± 2.42</td>
</tr>
<tr>
<td>Malondialdehyde, mmol/L</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; *, ** P < 0.05, 0.01 compared with before-vitamin E supplementation by paired t test.
TABLE 2
The effect of vitamin E supplementation on hydrogen peroxide production in lymphocytes of Asian men and women measured by flow cytometry1, 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (n = 26)</th>
<th>Male (n = 13)</th>
<th>Female (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before-vitamin E supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non PMA-stimulated</td>
<td>0.934 ± 0.002b</td>
<td>0.949 ± 0.004b</td>
<td>0.914 ± 0.006b</td>
</tr>
<tr>
<td>PMA-stimulated</td>
<td>1.343 ± 0.004a</td>
<td>1.283 ± 0.002a</td>
<td>1.400 ± 0.003a</td>
</tr>
<tr>
<td>After-vitamin E supplementation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non PMA-stimulated</td>
<td>0.565 ± 0.002c</td>
<td>0.557 ± 0.002c</td>
<td>0.573 ± 0.003c</td>
</tr>
<tr>
<td>PMA-stimulated</td>
<td>0.942 ± 0.003b</td>
<td>0.991 ± 0.002b</td>
<td>0.951 ± 0.003b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Those in a column without a common letter are different; *P < 0.05.
2 Intracellular free radical superoxide in lymphocytes is reduced by H2-DCFDA to H2O2, and recorded as green fluorescence (at 530 nm) of dichlorofluorescein (DCF). The amount of hydrogen peroxide (H2O2) is expressed as the mean fluorescence of dichlorofluorescein (DCF) and expressed as an arbitrary unit in this study.
3 Phorbol myristate acetate (PMA) is a potent inducer of H2O2 and was used as a positive control in this study.

The CD4/CD8 ratio is often used as an indicator in the detection and management of diseases. It provides information about the health of the individual. When the lymphocyte tests show a CD4/CD8 ratio < 1, it is a sign of possible viral infection or other causes of immunodeficiency. The relative proportion of lymphocyte subsets may be altered in certain disease states, such as depletion of the CD4 positive subset and elevation of CD8 T-cells during the course of HIV infection (Kidd and Vogt 1989). The findings from this investigation indicate that supplementation of healthy people with vitamin E can promote differentiation of total-T and T-helper subsets of T-lymphocytes and improve the CD4/CD8 ratio. Similar to our findings, Chavance et al. (1985) reported that healthy elderly French men with a blood vitamin E concentration 1.35 mg/L had higher CD4/CD8 ratios.

We found increased IL-2 receptor levels in the T-lymphocyte population of the individuals after vitamin E supplementation. The cytokine IL-2 is an important mediator required for T-lymphocyte proliferation. Mitogen-stimulated IL-2 production declines with age, subsequently affecting T-lymphocyte mediation (Meydani et al. 1998). Meydani and co-workers (1989, 1990 and 1998) found that supplementation of α-tocopheryl acetate at 400 or 800 mg/d increased IL-2 activity in lymphocytes obtained from elderly people after mitogen challenge. Our data indicated that there is a specific requirement for vitamin E in IL-2 receptor activation, total-T and T-helper cell proliferation, even in healthy individuals.

The plasma vitamin E concentration of our subjects before vitamin E supplementation was 14 μmol/L, which was considered deficient by Sauberlich et al. (1974). It has been suggested that the plasma vitamin E concentration of Cauca-

![FIGURE 1](https://academic.oup.com/jn/article-abstract/130/12/2935/4686300)

FIGURE 1 The effect of vitamin E supplementation on human lymphocyte proliferation in the presence or absence of phytohemagglutinin (PHA) and lipopolysaccharide (LPS) challenge. Isolated human lymphocytes were challenged before and after vitamin E supplementation with or without PHA (2.5 mg/L) or LPS (5.0 mg/L) for 48 h. Values are means and pooled SEM from men and women, n = 26. Values not sharing a common letter are significantly different, P < 0.05.

TABLE 3
The effect of vitamin E supplementation on immunological subsets of Asian men and women: measured by flow cytometry

<table>
<thead>
<tr>
<th>Lymphocyte subset (%)</th>
<th>Before vitamin E supplementation</th>
<th>After vitamin E supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-T (CD3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58.40 ± 1.95</td>
<td>62.80 ± 1.71*</td>
</tr>
<tr>
<td>Male</td>
<td>55.82 ± 2.23</td>
<td>61.91 ± 1.96*</td>
</tr>
<tr>
<td>Female</td>
<td>60.99 ± 3.12</td>
<td>63.69 ± 2.86*</td>
</tr>
<tr>
<td>T-Helper/Inducer (CD4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.10 ± 1.62</td>
<td>38.89 ± 1.35**</td>
</tr>
<tr>
<td>Male</td>
<td>31.79 ± 1.99</td>
<td>38.21 ± 1.81**</td>
</tr>
<tr>
<td>Female</td>
<td>36.41 ± 2.46</td>
<td>39.58 ± 2.06*</td>
</tr>
<tr>
<td>T-Suppressor/Cytotoxic (CD8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21.80 ± 0.75</td>
<td>21.70 ± 0.81</td>
</tr>
<tr>
<td>Male</td>
<td>21.30 ± 0.63</td>
<td>21.59 ± 1.01</td>
</tr>
<tr>
<td>Female</td>
<td>22.30 ± 1.37</td>
<td>21.80 ± 1.31</td>
</tr>
<tr>
<td>CD4/CD8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.59 ± 0.08</td>
<td>1.85 ± 0.09**</td>
</tr>
<tr>
<td>Male</td>
<td>1.50 ± 0.09</td>
<td>1.82 ± 0.13**</td>
</tr>
<tr>
<td>Female</td>
<td>1.69 ± 0.14</td>
<td>1.88 ± 0.13*</td>
</tr>
<tr>
<td>Natural killer (CD56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.55 ± 0.43</td>
<td>2.21 ± 0.32</td>
</tr>
<tr>
<td>Male</td>
<td>2.74 ± 0.73</td>
<td>2.11 ± 0.50</td>
</tr>
<tr>
<td>Female</td>
<td>2.35 ± 0.49</td>
<td>2.32 ± 0.43</td>
</tr>
<tr>
<td>Interleukin-2R (CD25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.05 ± 0.10</td>
<td>1.05 ± 0.10</td>
</tr>
<tr>
<td>Male</td>
<td>0.95 ± 0.11</td>
<td>1.04 ± 0.13</td>
</tr>
<tr>
<td>Female</td>
<td>1.15 ± 0.16</td>
<td>1.07 ± 0.17</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 26 for total (male and female), n = 13 for both male and female; *P < 0.05, 0.01 compared with pre-vitamin E supplementation by paired t test.
2 Ratio of T-helper and T-suppressor cells.
sians may be higher than that of Chinese (Benzie et al. 1998). The plasma vitamin E concentration in French men and women was reported to be 23.5 and 21.8 μmol/L, respectively (Hercberg et al. 1994). In Austrians, plasma vitamin E was 23.4 μmol/L (n = 59) (Ziouzenkova et al. 1996). Whether the difference in vitamin E concentration between Hong Kong Chinese and Caucasians is attributable to diet or to genetic factors cannot be answered by this investigation. Benzie et al. (1998) reported that a group of Caucasians in Hong Kong had significantly higher plasma vitamin E (33 μmol/L) compared with the Asian population (24 μmol/L) in the same environment.

The plasma vitamin E in our healthy subjects doubled after vitamin E supplementation at 400 μ/d for 28 d compared with baseline levels. With high doses of α-tocopherol (1200 μ/d) and longer supplementation periods (8 wk), up to a 187% increase of plasma level of α-tocopherol was reported in healthy people (Devaraj et al. 1996). Overall, vitamin E supplementation increased the antioxidant protective effect against both plasma lipid oxidation and DNA damage in our subjects. Both urinary 8-OHdG (P < 0.001) and the free radical lipid by-product plasma MDA (14%, P < 0.001) of the healthy subjects were lowered significantly after vitamin E supplementation. Wise et al. (1996) also reported decreased MDA in healthy subjects supplemented with capsules of dehydrated fruits and vegetables that are rich in vitamin E.

The urinary excretion rate of the damaged DNA adduct, 8-OHdG, has been proposed as an index of oxidative DNA damage rates in investigations on the relationships between oxidative stress–related DNA damage, aging and subsequent pathologic conditions (Backer and Weinstein 1980). In humans, it is postulated that DNA damage by free radicals is ~10⁶ base oxidation (cell · d) (Cadet and Berger, 1985). A small positive correlation was observed between urinary 8-OHdG and plasma MDA (r = 0.428, P < 0.01), suggesting that free radicals produced from plasma lipid peroxidation can damage the DNA molecules. Furthermore, it signifies that we can estimate the antioxidant effect of individuals in vivo simply by sampling urine, without having to withdraw blood.

In our study, the oxidative stress of T-lymphocytes decreased significantly by 44% after 28 d of vitamin E supplementation. Even when the lymphocytes were stimulated by PMA, the H₂O₂ concentration was much lower (35%) after vitamin E supplementation compared with before supplementation. A direct correlation between plasma vitamin E concentration and oxidative stress of lymphocytes was also recorded (r = −0.51, P < 0.01). A significant correlation (r = 0.33, P < 0.01) also was found between oxidative stress of lymphocytes (H₂O₂) and MDA. Whether the removal of oxidative species such as hydrogen peroxide by vitamin E helped improve the proliferation of lymphocytes of our subjects directly cannot be concluded at this point. The role of hydrogen peroxide in lymphocyte proliferation is controversial. On the one hand, free radicals are produced from cells of the immune system for defense against foreign bodies. On the other hand, excess free radicals can damage DNA and cell membrane integrity (Backer and Weinstein 1980). It has been suggested that in response to mitogens, phagocyte-released H₂O₂ could affect T cells by altering T-cell subset survival and allowing the function of a particular lymphocyte population such as T-helper cells to predominate (Staite et al. 1987). Los et al. (1995) observed that a high dose of hydrogen peroxide (>20 mmol/L) inhibited T-cell proliferation. However, small concentrations, between 10 and 20 mmol/L, significantly increased T-cell proliferation when applied for a short period under reducing conditions. These investigators (Los et al. 1995) and others (Marini et al. 1996) indicated that small amounts of reactive oxygen intermediates may in fact act as an important competence signal in T-lymphocytes, inducing early gene expression as well as cell proliferation.

In conclusion, short-term supplementation with vitamin E can modulate cell-mediated immunity and reduce oxidative stress in healthy Chinese men and women. The mechanisms involved in the oxidant-antioxidant balance of vitamin E required for the cellular proliferation of T cells in healthy individuals warrant further investigation.

LITERATURE CITED


