Vitamin E may affect the life expectancy of men, depending on dietary vitamin C intake and smoking

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Abstract

Background: antioxidants might protect against oxidative stress, which has been suggested as a cause of aging.

Methods: the Alpha-Tocopherol-Beta-Carotene (ATBC) Study recruited males aged 50–69 years who smoked at least five cigarettes per day at the baseline. The current study was restricted to participants who were followed up past the age of 65. Deaths were identified in the National Death Registry (1,445 deaths). We constructed Kaplan–Meier survival curves for all participants, and for four subgroups defined by dietary vitamin C intake and level of smoking. We also constructed Cox regression models allowing a different vitamin E effect for low and high age ranges.

Results: among all 10,837 participants, vitamin E had no effect on those who were 65–70 years old, but reduced mortality by 24% when participants were 71 or older. Among 2,284 men with dietary vitamin C intakes above the median who smoked less than a pack of cigarettes per day, vitamin E extended lifespan by 2 years at the upper limit of the follow-up age span. In this subgroup, the survival curves of vitamin E and no-vitamin E participants diverged at 71 years. In the other three subgroups covering 80% of the participants, vitamin E did not affect mortality.

Conclusions: this is the first study to strongly indicate that protection against oxidative stress can increase the life expectancy of some initially healthy population groups. Nevertheless, the lack of effect in 80% of this male cohort shows that vitamin E is no panacea for extending life expectancy.

Keywords: ageing, antioxidants, longevity, male, mortality, oxidative stress, randomised controlled trial, elderly

Introduction

Half a century ago, Harman proposed that endogenous free radicals might cause aging [1–3]. There is evidence that the level of oxidative stress increases during aging [4, 5], suggesting that its importance may be particularly great in the later phases of life. Yet, the role of oxidative stress in the aging of humans is not well understood.

A corollary of the oxidative stress theory is that antioxidants might increase lifespan because they protect against oxidants. Administration of antioxidants and overexpression of antioxidative enzymes, such as superoxide dismutase (SOD), catalase and glutathione reductase, have in some cases increased the lifespan of animals, but not uniformly. In Drosophila, overexpression of SOD increased lifespan in strains in which it was initially short, but not in strains in which lifespan was initially long [6]. Vitamin E administration [7] and overexpression of glutathione reductase [8] extended the lifespan of Drosophila during hyperoxia, but not while the oxygen level was normal. Administration of low levels of vitamin C [7] and vitamin E [9] increased lifespan of Drosophila, but high levels did not, indicating the importance of dosage. Finally, overexpression of SOD and catalase together extended the lifespan of Drosophila, whereas each alone had only a minor effect [10]. Heterogeneity in these findings seems to be particularly important since it indicates that antioxidant levels might be limiting factors under some conditions, although they are not a panacea for extending lifespan universally.

Vitamin E supplementation increased lifespan in some mice studies [11–13], but not in all [14, 15]; however, high levels of vitamin E reduced lifespan [12, 14]. Furthermore, vitamin C administration and overexpression of catalase increased the lifespan of mice in some studies [16–18], but...
Several large randomised trials of humans found that vitamin E supplementation does not reduce mortality [20–25]. These negative findings have often been interpreted as evidence that vitamin E does not protect against oxidative stress in humans.

The Alpha-Tocopherol-Beta-Carotene (ATBC) Study was a large randomised trial, which examined the effect of 50 mg/day of vitamin E on the risk of lung cancer in male smokers [25, 26]. In our previous analyses of the ATBC Study data, we found that age, smoking and dietary vitamin C intake significantly modified the vitamin E supplementation effect on the incidence of the common cold, pneumonia and tuberculosis [27–30]. This heterogeneity motivated us to test whether the effect on mortality might also be heterogeneous. We found significant modification of the supplementation effect in that vitamin E decreased mortality by 41% among those who were 66 or older at baseline and consumed vitamin C at a level above the median [31].

A large trial can accurately estimate the overall effect of vitamin E on mortality. However, if vitamin E influences the lifespan, it is possible that a benefit on the oldest participants might be camouflaged by the large middle-aged majority of study participants. In this study, we analyse the effect of vitamin E by the age of the participant at the follow-up. This allows us to accurately examine the age-dependency of vitamin E effect on the old ATBC Study participants. Since our focus is on the oldest participants, we restrict this analysis to the follow-up period when the participants were 65 and over.

**Methods**

**Participants**

The design and methods of the ATBC Study examining the effects of vitamin E (α-tocopheryl acetate, AT, 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung cancer and other cancers have been described earlier [25, 26]. The ATBC Study is registered at ClinicalTrials.gov under the identifier NCT00342992.

In brief, male participants aged 50–69 years had to smoke five or more cigarettes per day at entry to be eligible, and those enrolled in the trial (n = 29,133) were randomised to one of four intervention arms and administered placebo, AT, BC or AT + BC, using a 2 × 2 factorial design. Compliance with supplementation was high: some 90% of the participants took more than 90% of their prescribed capsules during their active participation in the trial; there were no differences in capsule consumption among the intervention groups [25, 26]. Compared with the baseline levels, supplementation increased the serum level of α-tocopherol by 50% [25, 26]. The intervention continued until 30 April 1993. The trial was approved by the institutional review boards and all participants gave written informed consent. This study was restricted to the participants who contributed to follow-up time after the age of 65, which left 10,837 men to this study. The lowest baseline age in this cohort was 56.7 years, and 1,805 participants had baseline age below 60 years.

**Baseline characteristics**

Before randomisation at the baseline, the men completed questionnaires on their medical and smoking histories and general background characteristics, and their weight was measured. A detailed dietary history questionnaire provided data regarding vitamin C and vitamin E consumption [32]. Dietary data were not available for 806 of the 10,837 participants.

**Outcome and follow-up time**

Deaths were identified in the National Death Registry as previously described [25]. Follow-up time for each participant began from the age of 65 or the day of randomisation (if it was over 65), and continued until death or the end of the trial (30 April 1993). The median follow-up time of the participants in the present analysis was 3.4 years, and there was a total of 57,773 person years of observation.

**Statistical models**

We estimated the effect of vitamin E supplementation on mortality through Cox regression models. We calculated the risk ratio (RR) and the 95% confidence interval (CI) of the RR using PROC PHREG in SAS (release 9.2, SAS Institute, Inc., Cary, NC, USA). We compared participants administered vitamin E (AT and AT + BC) with those not receiving vitamin E (the no-vitamin E participants; placebo and BC). As to supplementation, we carried out the analyses following the intention-to-treat principle. Because the deaths were identified in the National Death Registry which registers all deaths occurring in Finland, the loss-to-follow-up is insignificant.

To test whether the vitamin E supplementation effect is different at different age ranges, we first added a uniform vitamin E effect to the whole age range. Then we added separate vitamin E effects to early and late age regions [33]. The improvement of the Cox model fit was thereafter calculated from the change in $-2 \times \log \text{(likelihood)}$, which follows the $\chi^2(1 \text{ df})$ distribution. Several cut-off ages were tested, at 1-year intervals, around the region where the survival curves visually diverge in the figures. There were rather small differences between the models with the optimal cut point and models with cut point at the optimal $\pm 1$ year. In Figure 1, the cut point at 71 years led to $\chi^2(1 \text{ df}) = 5.06$, at 72 years led to $\chi^2(1 \text{ df}) = 6.47$ and at 73 years led to $\chi^2(1 \text{ df}) = 6.13$ improvement in the Cox model fit. In Figure 2A, the cut point at 69 years led to $\chi^2(1 \text{ df}) = 2.99$, at 70 years led to $\chi^2(1 \text{ df}) = 2.99$, at 71 years led to $\chi^2(1 \text{ df}) = 6.13$ and at 71 years
Figure 1. Effect of vitamin E supplementation on the lifespan of ATBC Study participants. There were 1,445 deaths among 10,837 men who contributed to the 37,773 person-years of observation after the age of 65 years. Kaplan–Meier survival curves for the vitamin E and no-vitamin E groups are shown. Each step indicates one death. The curves have been cut off at 77 years because the number of participants declined abruptly thereafter.

led to $\chi^2(1\ df) = 6.04$ improvement in the Cox model fit. Thus, the cut points at 72 and 70 years for Figures 1 and 2A, respectively, lead to the best Cox models. However, the cut point at 71 years leads to essentially the same improvements in the Cox models as the optimal cut points, and to simplify our presentation in the Results, we selected the cut point at 71 years for both Figures 1 and 2A.

Kaplan–Meier survival functions were constructed using STATA STS program (Release 9.1, Stata Corp, College Station, TX, USA). Two-tailed $P$-values were used.

Results

In all, 10,837 men contributed to the follow-up after the age of 65 years and there were 1,445 deaths during this period. There is no overall difference between the vitamin E and no-vitamin E groups when the entire survival curves are compared (Figure 1; $P = 0.3$ for the Cox model comparing the vitamin E and no-vitamin E groups). However, the survival curves for the vitamin E and no-vitamin E groups start to diverge at about 70 years. Therefore, we tested whether the addition of separate vitamin E effects to the early and late follow-up age ranges would improve the Cox model fit (see Methods).

The addition of separate vitamin E effects to the 65–70 years age range and for the ages over 71 significantly improves the statistical model ($\chi^2(1\ df) = 5.0$, $P = 0.03$). This model gives a RR of 1.00 (95% CI: 0.89–1.13; 1,113 deaths) for the 65–70 year age range, and RR = 0.76 (0.61–0.95; 332 deaths) for ages over 71. A constant relative effect on mortality transforms into a continuously increasing difference in lifespan by a higher age. At the end of the survival curves, the difference in the lifespan between the vitamin E and no-vitamin E groups is about 6 months (Figure 1).

Since we found previously that the effect of vitamin E on mortality was dependent on dietary vitamin C intake and the level of smoking [31], we constructed separate survival curves for the four groups defined by these variables (Figure 2).

The benefit of vitamin E was restricted to men who had dietary vitamin C intake above the median (90 mg/day) and smoked less than a pack of cigarettes per day at the baseline of the trial (Figure 2A). In this subgroup, the survival curves of the vitamin E and no-vitamin E participants start to diverge at the age of about 70 years. Adding separate vitamin E effects to Figure 2A for the 65–70 year range and to the ages over 71 years significantly improves the Cox model fit ($\chi^2(1\ df) = 6.0\ 0.02$). This model gives a RR of 0.91 (95% CI: 0.68–1.24; 169 deaths) for the 65–70 year age range, and RR = 0.43 (0.25–0.74; 61 deaths) for ages over 71. Vitamin E supplementation increased lifespan by about 2 years at the end of the survival curves in Figure 2A. The effect of vitamin E supplementation was not modified by β-carotene supplementation or dietary vitamin E intake (Table 1). We found previously that the effect of vitamin E on pneumonia incidence [28], but it did not modify the effect on mortality in this subgroup (Table 1).

In the other three participant groups of Figures 2B–D, defined by vitamin C intake and the level of smoking, there is no evidence that survival curves of the vitamin E and no-vitamin E participants diverge at about 70 years. Nevertheless, at the upper age range of each of these three figures, vitamin E participants lived slightly longer; however, the number of deaths at the upper age range is low (Figure 2B–D).

Discussion

In this study, we found that vitamin E supplemented participants had overall approximately half-a-year longer life expectancy at the upper limit of the follow-up age. This benefit of vitamin E was restricted to participants who smoked less than a pack of cigarettes per day and had vitamin C intake over the median.

Since dietary vitamin C intake has a close correlation with the daily amount of fruit and vegetables, the calculated vitamin C intake might be a proxy for fruit and vegetable intake. However, in our previous analysis, other substances in fruit and vegetables did not explain the modification of the vitamin E effect on mortality [31]. Furthermore, the synergism between vitamins E and C is well established. Vitamin E is the major lipid-soluble antioxidant that protects membranes against oxidative injury [34, 35]. In model systems, vitamin C reduces the oxidised form of vitamin E back to vitamin E [36–38], but the biological significance of this synergism is not well defined. Nevertheless, vitamin C
administration prevented the concomitant decreases in tissue vitamin E levels and body-weight gain of weanling guinea pigs administered oxidised frying oil [39] indicating that both vitamins together may be essential to protect against some forms of oxidative stress. Furthermore, smoking increases the plasma \( \alpha \)-tocopherol disappearance rate, which was normalised by vitamin C supplementation [40], and this physiological interaction between vitamins E and C and smoking gives a rationale for the relation between the extent of smoking, dietary vitamin C level and the effect of vitamin E supplementation in Figure 2. The biochemical findings were interpreted as evidence that higher doses of vitamins E and C might be beneficial for smokers in particular [40], whereas in Figure 2 the benefit of vitamin E was restricted to ATBC participants who smoked least.

The dependence of vitamin E supplementation effect on vitamin C intake level (Figure 2) implies that studies focusing on a single antioxidant might suggest a misleading conclusion about the potential roles of antioxidants. Furthermore, if the benefit of vitamin E is conditional on high vitamin C intake, it seems possible that combined vitamin E and C supplementation might affect the life expectancy of men belonging to the subgroup of Figure 2C.

Our findings are also important for the interpretation of the large randomised trials on vitamin E, which have mostly found no benefit from supplementation [20–25]. The large-scale vitamin E trials test the theory that cancers and cardiovascular diseases are caused by oxidative mechanisms. However, the average effect on mortality in a group of people with a wide age range does not measure the effect on lifespan. For example, although there is no overall difference between the survival curves of the vitamin E and no-vitamin E groups in Figure 1, the groups diverge at 71 years. The number of deaths in the 65–70 year age range (\( n = 1,113 \)) is substantially greater than in the ages over 71 years (\( n = 332 \)). If these two age ranges are analysed,

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**Figure 2.** Effect of vitamin E supplementation on lifespan in four subgroups according to dietary vitamin C intake and level of smoking. (A) 2,284 participants with \( \geq 90 \) mg/day vitamin C and 5–19 cigarettes per day, (B) 2,400 participants with \( \geq 90 \) mg/day vitamin C and \( \geq 20 \) cigarettes per day, (C) 2,245 participants with \(< 90 \) mg/day vitamin C and 5–19 cigarettes per day, (D) 3,102 participants with \(< 90 \) mg/day vitamin C and \( \geq 20 \) cigarettes per day. Vitamin C intake level was not available for 806 men, who are missing from these figures. Kaplan–Meier survival curves for the vitamin E and no-vitamin E groups are shown. Each step indicates one death. The curves have been cut off at 76.5 years because the number of participants declined abruptly thereafter.
It may be misleading to analyse large-scale trials with old people only from the time point of randomisation. Interaction between vitamins E and C may be physiologically relevant under some conditions. It may be misleading to analyse large-scale trials with old people only from the time point of randomisation.

### Key points

- Vitamin E may increase life expectancy of some groups of males.
- Interaction between vitamins E and C may be physiologically relevant under some conditions.
- It may be misleading to analyse large-scale trials with old people only from the time point of randomisation.

### Acknowledgements

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### Conflicts of interest

None declared.

### References

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#### Table 1. Effect of vitamin E on mortality among ATBC Study participants aged ≥71 years at follow up; men who had dietary vitamin C intake ≥90 mg/day and smoked 5–19 cigarettes/day

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of men</th>
<th>No. of deaths</th>
<th>RR (95% CI)</th>
<th>Vit E</th>
<th>No-vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>647</td>
<td>19</td>
<td>42</td>
<td>0.43 (0.25–0.74)</td>
<td></td>
</tr>
<tr>
<td>β-Carotene supplementation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>338</td>
<td>12</td>
<td>20</td>
<td>0.55 (0.27–1.13)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>309</td>
<td>7</td>
<td>22</td>
<td>0.31 (0.13–0.72)</td>
<td></td>
</tr>
<tr>
<td>Dietary vitamin E (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11.5</td>
<td>321</td>
<td>8</td>
<td>20</td>
<td>0.36 (0.15–0.80)</td>
<td></td>
</tr>
<tr>
<td>≥11.5</td>
<td>326</td>
<td>11</td>
<td>22</td>
<td>0.50 (0.24–1.04)</td>
<td></td>
</tr>
<tr>
<td>Age of smoking initiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>387</td>
<td>11</td>
<td>26</td>
<td>0.35 (0.17–0.71)</td>
<td></td>
</tr>
<tr>
<td>≥21</td>
<td>259</td>
<td>8</td>
<td>16</td>
<td>0.57 (0.24–1.32)</td>
<td></td>
</tr>
</tbody>
</table>

a In these subgroups, the vitamin E and no-vitamin E groups were of equal size within 14% accuracy.
b Cox proportional hazards model comparing participants who received vitamin E with those who did not. RR, risk ratio for death; CI, confidence interval.

The cut-off level for dietary vitamin E intake is at the median.
H. Hemilä and J. Kaprio


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220