COMMENTARY

Education

VITAMIN E AND ALL-CAUSE MORTALITY

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At the American Heart Association (AHA) annual meeting last November, the results of a vitamin E meta-analysis (Meta-Analysis: High-Dosage Vitamin E Supplementation May Increase All-Cause Mortality[1]) were announced. The *Annals of Internal Medicine* posted it on their website for early release, an action taken only when a study reports clinically urgent results.

This meta-analysis concluded that high-dose vitamin E, defined as ≥ 400 IU per day, increased all-cause mortality by 4%, which was small but statistically significant (P=.035). On the other hand, vitamin E doses < 200 IU had no significant effect on all-cause mortality.

The authors analyzed 19 previously published clinical trials consisting of 135,967 volunteers, and the inclusion criteria were as follows: participants in the studies had to be men or non-pregnant women randomized to take vitamin E alone or in combination with other vitamins or minerals, and the studies had to have a control or placebo group, last longer than 1 year, and have reported at least 10 deaths in the trial. During the clinical trials, from 16.5 to 2000 IU per day of vitamin E was administered.

The authors' conclusion that high-dose vitamin E increases all-cause mortality is alarming and surprising, especially since three previous meta-analyses found no evidence that vitamin E supplementation of up to 800 IU/day significantly increased or decreased cardiovascular disease (CVD) mortality or all-cause mortality.[2-4] A closer look at the current meta-analysis reveals a seriously flawed study.

DISPARITIES AMONG TRIALS

A meta-analysis is only as strong as the data combined for analysis. Randomized, controlled trials are done on different populations and subgroups representing various patients characteristics. Since different subgroups may respond to the same therapy differently, combining very heterogeneous studies can make the analysis unreliable.[5] A meta-analysis should analyze data from homogenous populations using similar study protocols. Not only did this meta-analysis not meet these criteria for a high-quality study, it also conflicts with prior meta-analyses on vitamin E use in CVD patients and epidemiological data on the benefits of vitamin E.

There were 44,168 volunteers in the high-dose vitamin E studies. The studies that tested high-dose vitamin E looked at widely divergent populations. Five studies enrolled volunteers at risk for, or diagnosed with CVD. Even within these trials there was significant heterogeneity. The Cambridge Heart Antioxidant Study (CHAOS)[6] and the Women's Angiographic Vitamin and Estrogen (WAVE)[7] and Heart Outcomes Prevention Evaluation (HOPE)[8] studies enrolled volunteers with angiographically-confirmed coronary atherosclerosis, but WAVE participants were restricted to postmenopausal women.

The final two cardiovascular trials, accounting for nearly half of all participants in the high-dose vitamin E group, analyzed the use of vitamin E in volunteers at substantially greater risk of mortality than the previous three studies. The Medical Research Council/British Heart Foundation Heart Protection Study (MRC/BHF HPS)[9] enrolled volunteers who "were considered to be at substantial 5-year risk of death from coronary heart disease, of other occlusive arterial disease, or diabetes mellitus, or of treated hypertension alone." The Secondary Prevention with Antioxidants of Cardiovascular Disease in End-stage Renal Disease (SPACE)[10] trial also enrolled people with CVD, but had the added complication of all the participants having end-stage renal failure and being on dialysis.

The remaining 5 clinical trials, which accounted for the other 50% of the participants in the high-dose vitamin E trials, evaluated the use of vitamin E in volunteers with physiologically disparate illnesses. The Roche European American Cataract Foundation Heart Protection Study (REACT)[11] and Vitamin E, Cataracts, and Age-Related Maculopathy (VECAT)[12] investigations studied men and women with age-related cataracts, while the Alzheimer's Disease Cooperative Study (ADCS)[13] and the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP)[14] trial enrolled Alzheimer's and Parkinson disease

patients, respectively. The Polyp Prevention Study (PPS)[15] participants all had a recent history of large bowel adenoma. The differences between study populations make it difficult to arrive at clinically meaningful conclusions.

There was also a high degree of heterogeneity of study protocols. Five of the 11 high-dose studies, accounting for 27,025 participants (or 61% of the total high-dose study population), administered vitamin E in conjunction with other supplements. Deleterious effects from the other supplements cannot be ruled out.

In the Age-Related Eye Disease Study (AREDS)[16] clinical trial, which accounted for 18% of the total high-dose vitamin E participants, 400 IU of vitamin E per day was given along with 500 mg vitamin C, 15 mg beta-carotene, 80 mg of zinc, and 2 mg copper. This is twice the upper limit (UL) of safety for zinc. Zinc can compete with copper for absorption and lead to copper deficiency at doses as low as 18.5 or 26 mg per day.[17] Excessive zinc consumption can cause copper deficiency, and although copper was also given, it was in the insoluble and unabsorbable cupric oxide form. Even if the copper were absorbed, however, the low dose of copper given in this study was probably not enough to overcome the loss from such a high dose of zinc. This may account for the non-significant increase in mortality in this trial.

Beta-carotene, which was used in 4 of the high-dose trials, may also have contributed to their findings. There are more than 600 naturally occurring carotenoids, of which beta-carotene is just one. Synthetic beta-carotene is not structurally the same as naturally occurring beta-carotene and can have deleterious effects.

What's in a Title?

Aside from the fact that that many of the clinical trials analyzed in this meta-analysis did not administer vitamin E alone, it can be argued that the clinical trials did not even test "vitamin E." This is a matter of definition and is clinically relevant.

The distinction here is one of natural versus synthetic vitamin E. Natural vitamin E is a mixture of 8 chemicals—four tocopherols (alpha, beta, delta, and gamma) and four tocotrienols (also alpha, beta, delta, and gamma). These forms occur together and in various concentrations in different foods. Supplementation with just alpha-tocopherol, in contrast to mixed tocopeherols, can decrease plasma gamma-tocopherol concentrations by 30-50%,[18] potentially creating an imbalance in the body's antioxidant system.

Whereas all fractions of natural vitamin E are in the RRR racemic form, synthetic vitamin E is a mixture of 8 stereoisomers, only one of which is analogous to the naturally occurring RRR alpha tocopherol. The other seven stereoisomers are not native to our diet and have a potentially antagonistic influence on the natural RRR alpha tocopherol form.

Only one trial in the high-dose group, the HOPE study, claimed that it used "natural vitamin E." In all other trials that reported the form of vitamin E used, synthetic vitamin E was tested. In the WAVE study, not only was the form of vitamin E not reported, the researchers were unsure of which form of vitamin E had been used. They had to contact the manufacturer to find out,[19] and it was determined that they used synthetic vitamin E.

This is disconcerting because it suggests that neither the researchers nor the staff of the journal in which their data were published believed there were any important differences between natural and synthetic vitamin E. However, there is a growing body of evidence that form does matter. Mixed tocopherols in the proportions found in food and gamma-tocopherol alone had greater anti-platelet aggregation and antioxidant effects than alpha-tocopherol in animal studies.[20,21] Mixed tocopherols also decreased lactic acid dehydrogenase (LDH) in experimental hypoxia-reoxygenation injury of rat myocytes.[21] The concentration of superoxide dismutase (SOD) in these studies was increased significantly more by gamma-tocopherol and mixed tocopherols than by alpha-tocopherol alone ($P < .05$ in both studies).

Human observational *ex vivo* and *in vivo* trials have also revealed important differences between subfractions of vitamin E. In patients with coronary artery disease, gamma-tocopherol is reduced, but alpha-tocopherol is not reduced.[22] An *ex vivo* study on human macrophages revealed that gamma-tocopherol can inhibit the cyclooxygenase-2 (COX-2) enzyme to a greater extent than alpha-tocopherol.[23] In an *in vivo* trial supplementing with mixed tocopherols and alpha-tocopherol for 8 weeks, superoxide dismutase (SOD) and endothelial constitutive nitric oxide synthase (ecNOS) significantly increased in the mixed tocopherol group, compared to alpha-tocopherol alone ($P < .01$).[24]

The form of vitamin E used in the SPACE trial may have accounted for its non-significant increase in mortality in the treatment group compared to placebo. The population studied in the SPACE trial was patients with cardiovascular disease and end-stage renal failure who were on hemodialysis. In a previous study, patients on dialysis with end-stage renal disease who were not supplementing with vitamin E were observed to have greater serum gamma-tocopherol compared to healthy controls (3.17 ± 0.37 μg/mL vs 1.08 ± 0.06 μg/mL, respectively; $P < .0001$).[25] Supplementation with gamma-enriched tocopherols, but not alpha-tocopherols, in this same group of volunteers lowered median C-reactive protein (CRP) significantly in hemodialysis patients (4.4 to 2.1 mg/L; $P < .02$). And as previously mentioned, supplementing with alpha-tocopherol

alone can reduce gamma-tocopherol levels by 30-50%. In the SPACE trial, supplementation with alpha-tocopherol may have decreased gamma-tocopherol levels and acted as a pro-oxidant, thereby contributing to the study's results.

The Role of Vitamin E in the Body

When separated from their complexes, vitamins cannot be expected to perform their specific functions. Similarly, clinical trials that do not provide all the nutrients required for proper biochemical functioning cannot be expected to yield optimal results. When isolated into artificial commercial forms, like alpha-tocopherol, unintended consequences may result. A major criticism of using alpha-tocopherol or other non-natural forms of vitamin E is that it may act as a pro-oxidant.

The principle function of vitamin E is the maintenance of cellular membrane integrity through its free-radical quenching potential.[26] However, vitamin E can only maintain its antioxidant status if all other molecules necessary to convert the tocopherol back to its fully reduced state are also replete. In the process of neutralizing free radicals, the tocopherols become tocopheryl radicals. These free radicals can be reduced back to their tocopherol form by vitamin C, coenzyme Q10, and reduced glutathione (GSSH), although reduction by vitamin C is probably the most active pathway.[26] In turn, GSSH, a tripeptide, requires selenium and riboflavin (vitamin B_1) to cycle through its redox pathway.

Vitamin C, once oxidized in the process of regenerating tocopherol, must itself be regenerated. Two molecules of GSSH are used to reduce ascorbic acid back to dehydroascorbic acid, leaving oxidized glutathione (GSSG) behind. In turn, GSSG is converted back into GSSH by the electron-donating potential of NADPH, a niacin-containing complex.

The depletion or absence of any of these nutrients results in a decreased capacity to regenerate tocopherol. The tocopherol radical will increase, as will the oxidative stress on cells. Notably, it has previously been demonstrated that total GSSH and plasma glutathione peroxidase activity were significantly reduced in patients with moderate ($P < 0.01$) and severe ($P < 0.001$) chronic renal failure.[27] While the clinical trials analyzed in this meta-analysis did not individually show clinically significant increases in mortality, the potential clinical benefit from vitamin E may have been reduced if the populations studied were not replete with these other antioxidants.

Conclusion

The conclusion that high-dose vitamin E increases all-cause mortality is simply not supported by this meta-analysis. The clinical trials pooled for this study used heterogeneous populations and study protocols. Synthetic vitamin E was used, mostly in combination with other supplements, which makes it difficult to conclude that vitamin E was *the* agent that caused an increase in mortality. Additionally, alpha-tocopherol can deplete gamma-tocopherol and, in illnesses where endogenous antioxidants are decreased, alpha-tocopherol may become a pro-oxidant.

While the conclusions by the authors of this meta-analysis should not deter people from supplementing with vitamin E, a rational approach to supplementation, and a more rational approach for the development of clinical trials, needs to be developed. Supplementation regimens should ensure an adequate intake of a mixture of naturally-derived substances and should be just that—supplements. They should not take the place of optimizing dietary intake of healthy nutrients.

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