COMMENTARY

A RATIONAL APPROACH TO ANTIOXIDANT THERAPY AND VITAMIN E

Mark Hyman, MD, Joseph Pizzorno, ND, Andrew Weil, MD

Interpreting Studies: The Importance of Context and the Role of the Scientist

The recent meta-analysis1 and media coverage surrounding the risks and benefits of vitamin E repeat a common failure of physicians and media—the sensationalization of medical research reports combined with the lack of context and nuance that leaves practicing physicians, patients and consumers confused. Fortunately a more dispassionate and rational view placing the current meta-analysis in the context of a broader literature on vitamin E and antioxidants can be a better guide to recommendations and policy.

Medical science is imprecise and the tools of analysis and research are imperfect despite our best intentions. No statistical model or research methodology can accurately predict the outcome in all humans who vary greatly in habits and genes. Medicine has in recent years been humbled in its hunger to help by its mistakes including hormone replacement therapy,[2] which in the end hurt rather than helped the heart, the "lipids only hypothesis" of cardiovascular disease that ignores the important role of inflammation,[3] and the belated,[4] sober end to our love affair with COX-2 inhibitors.

Uncertainty is common in medicine. In the fact of uncertainty, we must take a broader view, step back from the canvas of individual studies and meta-analyses and view the landscape of medicine and scientific inquiry as a whole, while understanding the limitations and value of different tools of research. We require *all* the tools in order create sense and be sensible in a shifting sea of data points. The goal of seeking an informed balanced overview is to help us create understanding from knowledge, to sort through the facts and place them in the context of biologic principles, against the backdrop of all we know. Then we ask ourselves if the new data fit into the landscape or appear like a polar bear in a desert. We must also recognize that the questions we ask, how we ask them, and why, all inform the answers we receive.

The responsibility of the scientist is to filter the research, place it in context, provide hope where appropriate and caution where necessary, but most importantly to be an informed, measured guide to a public seeking to gain health and ameliorate suffering. What can we learn from the recent example of vitamin E? There are a number of limitations from the recent vitamin E meta-analysis and opportunities to refocus our research strategy. Using long-term randomized trials and meta-analyses for the study of lifestyle interventions, diet and individual nutrients is fraught with difficulty in design and effective separation of the plethora of their biologic effects. We may, in fact, be asking the wrong question. Asking if high dose vitamin E is helpful or harmful may not provide the information we seek. We may better ask if restoring normal redox balance is protective, and if so, how can we best achieve that goal? Nutrients, in general, do not lend themselves to a research design that is best suited for pharmacological interventions because, unlike pharmaceuticals, nutrients are multi-functional substances built into our evolutionary design. There are many confounding factors in any randomized trial and meta-analysis. The vagaries in individual diets, habits, pre-clinical and clinical disease, genomic diversity of SNPs (single nucleotide polymorphisms), the bioavailability and biological specificity of different forms of individual nutrients, and selection of the most effective combinations of nutrients, make nutritional research through randomized trials and meta-analyses very problematic. Given that a methodologically sound, definitive randomized placebo-controlled trial has not been done and may never be done, the best we can currently do is take the best available evidence from basic science, studies of redox status, nutrient levels, trials with clearly measurable intermediate endpoints, relevant biomarkers, gene expression analysis, along with observational data and large-scale randomized trials and then synthesize all the points of evidence into a biologically plausible theory, clinical guidelines and when justified public policy. This was not done.

Meta-analysis confronts us with many methodological difficulties including publication bias; heterogeneity of the trials; variations in quality, design and purpose of the individual trials, as well as the fact that the findings are often not borne out in later randomized trials on the same subject.[5] LeLorier admonishes us that "the popularity of meta-analysis may at least partly come from the fact that it makes life simpler and easier for reviewers as well as readers. However, oversimplification may lead to inappropriate conclusions." Furthermore, conclusions of many studies noted in abstracts of major journals are at odds with data published in the same paper.[6] This oversimplification and conclusions inconsistent with the data were evident in the recent-metanalysis on vitamin E.

Reviewing the Review: Errors in Design and Interpretation

With these caveats in mind, a brief examination of the basic science, observational data, randomized trials and interventional studies with clear intermediate endpoints can provide a more rational context and framework for recommendations about vitamin E,[7] as well as a model for assessing the role of nutrients and other biological therapies in health and disease.

The data in this meta-analysis pooled 135,967 subjects from 19 studies, which individually did not show any statistically significant increase in mortality. The 19 studies were selected from 2,170 studies. The authors concluded, "high dose (>400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided." However the authors in the body of the paper provide ade-

quate caution about over interpretation of the results. They properly raise concerns related to publication bias; the small size of most of the included trials; the inclusion of patients with chronic disease limiting relevance to healthy adults; the concomitant use of other vitamins including beta-carotene that has shown an increase in morality in smokers and drinkers; the lack of homogeneity of tocopherol isomers among studies (inconsistent use of synthetic all-rac alpha tocopherol or naturally occurring RRR-alpha-tocopherol); and the non-statistically significant reduction in mortality from doses less than 200 IU. The benefits for intermediate endpoints in many of the trials cited (Cambridge Heart Antioxidant Study [CHAOS],[8] SPACE[9]) such as the reductions in nonfatal myocardial infarction (MI) by 70% to 77% were not discussed. These limitations were not included in media coverage, nor taken into consideration by many of the expert commentators on the results.

Other shortcomings of the meta-analysis by Miller et al, are highlighted by a more careful review of the three flawed larger trials on which the conclusion that high dose vitamin E supplements have a relative risk of death of 1.04 is based. These were the MRC/BHF[11] Heart Protection Study, CHAOS,[8] and AREDS[12] (Age-Related Eye Disease Study) Report No. 9, which represented two-thirds of the participants in the high-dose trials. The data of the other trials is less than convincing, and the interpretation and selective omission of key findings of the CHAOS, AREDS and MRC/BHF trials deserve closer scrutiny because they serve as the primary justification for the conclusions that vitamin E increases mortality. Of the 19 trials, 11 showed reduction in mortality, including 7/8 low-dose and 4/11 high-dose trials (which comprised only 30% of patients in the entire meta-analysis). It is also important to note that the all cause mortality was available, except for one study, with combined high and low doses and that for the purposes of the meta-analysis, they doses were averaged. In addition, the statistical significance of the data is marginal. In the 11 high dose trials, the risk difference was 39 per 10,000 persons, but the confidence interval was only 3 to 74 per 10,000 persons and the risk ratio was 1.04 with a confidence interval of only 1.01 to 1.07 indicating that the risk may have been close to zero, even in the high dose trials.

The CHAOS investigators assessed the effect of RRR-alpha-tocopherol at 400 or 800 I.U. in 1,035 patients with ischemic heart disease (967 more received a placebo) for 510 days. The vitamin E supplementation reduced the risk of non-fatal MI by 77%. There was a small statistically non-significant increase in cardiovascular deaths in the treatment group; however, those patients were sicker at baseline, the deaths occurred very early in the study, and further analysis revealed that those patients were actually non-compliant with the vitamin E. Later mortality estimates from CHAOS came from a research letter, not a peer-reviewed study, 18 and included data after the study was officially ended, and thus subject to information bias due to lack of complete reporting.

The single largest trial, the MRC/BHF Heart Protection Study, of 20,536 high-risk individuals using synthetic or racemic-alpha-tocopherol of 600mg a day combined with 250mg of vitamin C and 20mg of beta-carotene (known to increase cancer risk in high-risk individuals). The authors concluded that the results "effectively ruled out any substantial reductions, or, indeed, increases, in heart attacks, strokes, cancers, or other major adverse events during 5 years of use of these vitamins." Any benefit might have been mitigated by the use of the potentially harmful synthetic beta-carotene, and the synthetic racemic-alpha-tocopherol. While the study did measure serum levels of antioxidants, redox status was not assessed, leaving open the question of whether the anti-oxidants achieved their expected biologic effects.

The AREDS trials are multiple and the selection of the negative trials for inclusion in the analysis is perplexing despite publication of conflicting results in the same issue of the *Archives of Ophthalmology*. Report No. 9,[12] included in the meta-analysis, showed no benefit in reduction of cataracts from high dose combined anti-oxidant therapy with beta-carotene 15mg, vitamin C 500mg, and vitamin E 400 IU either alone or combined with high-dose zinc 80mg or copper 2mg (using an insoluble, non-absorbed form cupric oxide). This is not unexpected because the primary mechanism of cataract formation appears to be glycation, not oxidation. Yet an analysis of different endpoints from the same data in Report No. 8[14] showed a 34% reduction in age-related oxidative stress mediated macular degeneration in 3,640 enrolled study participants, aged 55-80 years, over 6.3 years. No statistically significant serious adverse effect was associated with any of the formulations in either analysis. Report No. 11[15] estimated that if all persons at risk for AMD (8 million) were to receive the antioxidants in the AREDS trial, the 300,000 of the 1.3 million at high risk for advanced AMD would avoid that fate and provide considerable public health benefits.

The studies cited either showed benefit in non-fatal intermediate endpoints such as MI, macular degeneration, arteriosclerosis, angina, prostate and colon cancer, or at the very least showed no harm. The conclusions in many studies do not accurately reflect the data. In the WAVE[16] trial of hormone replacement and antioxidant interventions in post-menopausal women with coronary artery disease, the antioxidant treatment group was sicker, had more smokers and hypertensives, and less patients taking protective treatments with angiotensin converting enzyme inhibitors or aspirin, so it is not surprising that the vitamin treatment group showed a statistically non-significant trend toward worse cardiovascular outcomes. The authors found that neither the hormone nor antioxidant group showed benefit and suggested harm from both therapies as noted in the conclusion; however, the lead author of the study, in a reply to a letter to the editor, conceded that, "these results do not prove that high-dose vitamin C and E supplements are harmful."[17]

The meta-analysis did not put its results in perspective by reviewing the context of research on vitamin E including the many positive observational,[18,19] and interventional studies,[20] a large body of basic science research and careful analysis of endpoints other than total mortality from the studies in the meta-analysis. It is important to note that many of the larger randomized trials showed statistically insignificant or barely significant benefit or harm. Also randomized controlled trials on vitamin E and cardiovascular disease were subjected to meta-analysis in three other studies;[21-24] and no significant increase in cardiac or all cause mortality was found with doses of up to 800 IU per day. If vitamin E or the antioxidant theory is useful in preventing and treating disease why do we not see a larger effect? Is it that this question cannot be

answered through randomized trials because of the enormous methodological challenge, or is it that we must revisit the proposed value of anti-oxidants in secondary prevention? Any new study should be considered in the context of the best available evidence from animal studies, basic science, and observational and small and large interventional trials.

The Role of Vitamin E in Health and Disease

The data on vitamin E collectively point to potentially important beneficial biologic effects that are at odds with the reported mortality data. While the basic science must also be cautiously used in making clinical assumptions, there are many reasons to hypothesize a useful role of vitamin E in promoting health as well as preventing and treating disease. A recent review on vitamin E outlined evidence that inhibits LDL oxidation, acts on coagulation, platelet aggregation and endothelial aggregation, down-regulates cellular adhesion molecules, inhibits release of IL-1 beta from lipopolysaccharide activated monocytes, inhibits protein kinase C affecting a broad array of cell signaling molecules and reduces inflammation and smooth muscle cell proliferation, induces apoptosis, and enhances cell mediated immunity.[21] Non-antioxidant activities of vitamin E include its influence on the activity of several other enzymes (eg, PP2A, COX-2, 5-lipooxygenase, nitric oxide synthase, NADPH-oxidase, superoxide dismutase, phopholipase A2) and it also modulates the expression of genes that are involved in atherosclerosis (eg, scavenger receptors, integrins, selectins, cytokines, cyclins).[25] There are clinical trials indicating that vitamin E normalizes autonomic tone in diabetics,[26] reduces risk for macular degeneration,[12] prostate, and colon cancer,[27] reduces MI, retards arteriosclerosis,[26] and slows progression of neurodegenerative disease[27] as well as showing benefits in intermittent claudication, fibrocystic breast disease, premenstrual syndrome, osteoarthritis, and infertility.[30] Because vitamin E is actually a mixture of eight compounds (alpha, beta, gamma and delta tocopherols and alpha, beta, gamma and delta tocotrienols), and because they are an integral part of cell signaling mechanisms, and the integrated redox system including ascorbate, carotenoids, r-lipoic acid, and glutathione, the ideal dosage, form and blend of vitamin E and other antioxidants and cell signaling molecules is needed to clearly determine benefit or harm.

Responsibility in Science and the Media

Unfortunately answering these questions through randomized clinical trials may not be feasible because of the cost, time and multiple variables required. We cannot assume that testing all nutrients as drugs in randomized clinical trials even using a single agent with a single variable will be feasible or if done will provide us with useful information with adequate external validity. Understanding the role of nutrients as part of the complex array of biologic response modifiers found in our diet and our environment should eventually bring more clarity and guidance for recommendations.

Employing the linear reductionist model using varying study designs raises many questions regarding the validity of the data. Are the data in a population of patients with pre-existing cardiovascular, diabetes and other chronic illness applicable to a healthy population interested in prevention? Are results based only alpha tocopherols (racemic and RRR, synthetic and natural) relevant when diets contain mostly gamma tocopherols, and the frequently used racemic form containing l-alpha tocopherol may inactivate RRR-alpha-tocopherol? How do the other forms of antioxidants and nutrients used in the studies affect the redox balance, signaling and gene expression that determine harm or benefit? How do gene polymorphisms play a role in nutrient effects? Did the high-frequency polymorphism of endolethial nitric oxide synthase (eNOS) in the English patients in the CHAOS trial lead to the 77% reduction in non-fatal MI with vitamin E by improving endothelial function? Did differences in pre-treatment nutrient levels because of geographic location, dietary preferences and nutritional status impact outcomes? Would patient stratification by clinical profile and assessment of biomarkers of vitamin E action including effects on redox status, endothelial function, immune function, cell signaling and gene expression have provided insight on the possible risks and benefits with different population groups?

New data is best interpreted in the light of existing recommendations and findings of independent and governmental bodies designed to guide policy. The recommendations from the Institute of Medicine,[31] the US Preventive Services Task Force,[32] the Lewin Group,[33] and literature reviews published in the *Journal of the American Medical Association*[34] and the *New England Journal of Medicine*[35] on vitamin therapy all contradict the findings of this meta-analysis. In fact, an independent analysis of the data on the clinical and economic impact of vitamins in health disease by the Lewin Group for Wyeth Consumer Healthcare found that the potential savings from multi-vitamin use in the Medicare population was $3.9 billion over 5 years from a reduction in cardiovascular and improved immune function and reduction in colon caner with no attributable risk.

In the light of this complex set of data it is inappropriate for the media to publicize isolated findings of selected studies and propagate premature conclusions.[36] It is even more concerning when the academic or practicing physician does not bring an appropriately open mind to consideration of complex biologic phenomena, namely oxidative stress in health and disease, and thereby avoid measured, thoughtful and dispassionate discussion of any and all interventions related to this possible risk factor, whether natural or pharmacologic. Our duty is to educate, inform and provide guidance, not reflexively react to the shifting sands of scientific discovery or fail to understand the importance of considering new findings in the context of all levels of data and applying the rule of reasonableness.

—Mark Hyman, MD
Editor-in-Chief
Alternative Therapies in Health and Medicine

—Joseph Pizzorno, ND
Editor
Integrative Medicine: A Clinician's Journal

—Andrew Weil, MD
Program for Integrative Medicine
University of Arizona

References

1. Miller ER III, Pastor-Barriuso R, Dalal D, et al. Meta-Analysis: High-Dosage Vitamin E Supplementation May Increase All-Cause Mortality. *Ann Intern Med*. 2004 Nov 10
2. Rossouw JE, Anderson GL, Prentice RL, et al. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. 2002 Jul 17;288(3):321-333.
3. Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. *Am Heart J*. 2004;148:S19-S26.
4. Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA*. 2001 Aug 22-29;286(8):954-959. Review.
5. LeLorier J, Gregoire G, Benhaddad A, Lapierre J, Derderian F. Discrepancies between meta-analyses and subsequent large randomized, controlled trials. *N Engl J Med*. 1997;337(8):536-542.
6. Pitkin RM, Branagan MA, Burmeister LF. Accuracy of Data in Abstracts of Published Research Articles, *JAMA*. 1999; 281:1110 - 1111.
7. Williams R. Biochemical individuality. New York: John Wiley and Sons, 1963.
8. Neuzil J,Weber C, Kontush A. The role of vitamin E in atherogenesis: linking the chemical, biological and clinical aspects of the disease. *Atherosclerosis. 2001;157:257–283.*
9. Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet*. 1996;347:781–786.
10. Boaz M, Smetana S, Weinstein T, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo controlled trial. *Lancet*. 2000; 356: 1213–1218.
11. Heart Protection Study Collaborative Group MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002; 360: 23–33.
12. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. *Arch Ophthalmol*. 2001;119(10):1439-1452.
13. Mitchinson MJ, Stephens NG, Parsons A, et al. Mortality in the CHAOS trial. *Lancet.* 1999 Jan 30;353(9150):381-382.
14. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119(10):1417-1436.
15. Bressler NM, Bressler SB, Congdon NG, et al. Age-Related Eye Disease Study Research Group. Potential public health impact of Age-Related Eye Disease Study results:AREDS report no. 11. *Arch Ophthalmol*. 2003;121(11):1621-1624.
16. Waters DD, Alderman EL, Hsia J, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. *JAMA*. 2002;288:2432-2440.
17. Waters D. Vitamins and Hormone Therapy for CAD. *JAMA*. 2003;289:982.
18. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med.* 1993 May 20;328(20):1444-1449.
19. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*. 1993 May 20;328(20):1450-1456.
20. Salonen RM, Nyyssonen K, Kaikkonen J, et al. Antioxidant Supplementation in Atherosclerosis Prevention Study.Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression: the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study. *Circulation*. 2003 Feb 25;107(7):947-953.
21. Brigelius-Flohe R, Kelly FJ, Salonen JT, et al. The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr.* 2002 Oct; 76(4):703-716.
22. Shekelle PG, Morton SC, Jungvig LK, et al. Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease. *J Gen Intern Med*. 2004;19(4):380-389.
23. Eidelman RS, Hollar D, Hebert PR, Lamas GA, Hennekens CH. Randomized trials of vitamin E in the treatment and prevention of cardiovascular disease. *Arch Intern Med*. 2004;164(14):1552-1556.
24. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet*. 2003;361(9374):2017-2023.
25. Munteanu A, Zingg JM, Azzi A. Anti-atherosclerotic effects of vitamin E--myth or reality? *J Cell Mol Med*. 2004 ;8(1):59-76.
26. Manzella D, Barbieri M, Ragno E, Paolisso G. Chronic administration of pharmacologic doses of vitamin E improves the cardiac autonomic nervous system in patients with type 2 diabetes. *Am J Clin Nutr*. 2001 Jun;73(6):1052-1057.
27. Virtamo J, Pietinen P, Huttunen JK, et al . ATBC Study Group. Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. *JAMA*. 2003 Jul 23;290(4):476-485.
28. Fang JC, Kinlay S, Beltrame J, Hikiti H, Wainstein M, Behrendt D, Suh J, Frei B, Mudge GH, Selwyn AP, Ganz P. Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet*. 2002. 30;359(9312):1108-1113.
29. Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. N Engl J Med. 1997; 336:1216-1222.
30. http://www.pdrhealth.com/drug_info/nmdrugprofiles/nutsupdrugs/vit_0266.shtml
31. http://www.iom.edu/project.asp?id=4574
32. Morris CD, Carson S. Routine vitamin supplementation to prevent cardiovascular disease: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2003 Jul 1;139(1):56-70. Review.
33. The Lewin Group. A Study of the Cost Effects of Daily Multivitamins for Older Adults, Prepared for Wyeth Consumer Healthcare, January 14, 2004.
34. Fairfield KM, Fletcher RH. Vitamins for Chronic Disease Prevention in Adults: Scientific Review JAMA, Jun 2002; 287:3116 - 3126.
35. Willett WC, Stampfer MJ. What Vitamins Should I be Taking Doctor? *N Engl J Med*. 2001; 345:1819-1824.
36. Moynihan, R, Bero, L, Ross-Degnan, D, Coverage by news media of the benefits and risks of medications. *N Engl J Med*. 2000 Jun1;342(22):1645-50.